These records are from CDER's historical file of information previously disclosed under the Freedom of Information Act (FOIA) for this drug approval and are being posted as is. They have not been previously posted on Drugs@FDA because of the quality (e.g., readability) of some of the records. The documents were redacted before amendments to FOIA required that the volume of redacted information be identified and/or the FOIA exemption be cited. These are the best available copies.

Summary Basis of Approval
Cover Form

Appl #: 020690

Firm: EISAI AMERICA
Reviewing Div: 120
Trade Name: ARICEPT (DONEPEZIL HCL) ORAL TABS 5/10MG
Generic Name:

DONEPEZIL HCL

Approval Letter:	Y	Statistician Review:	N
SBA Form:	N	Bio/Dissolution Review:	Y
Final Printed Labeling:	N	Microbiologist Review:	N
Medical Officer Review:	Y	NAS/NRC Review:	N
Chemist Review:	Y	Pharmacologist Review:	Y
Federal Register Notice:	N	Completion Date:	11 APR 97

20-690

ARICEPT

Approval Letter And Related Correspondence

DEC 4 1996

ARICEPT™
(Donepezil HCl) Tablets

NDA 20-690

Document History

Volume 1 of 5

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-690

Eisai America, Inc.
Attention: Nicholas J. Farina, Ph.D.
Glenpointe Centre East
300 Frank W. Burr
Teaneck, NJ 07666-6741

NOV 25 1996

Dear Dr. Farina:

Please refer to your March 29, 1996 new drug application submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for Aricept™ (donepezil hydrochloride) 5 mg and 10 mg Tablets.

We acknowledge receipt of your amendments dated October 3, 1996, October 11, 1996, October 26, 1996 and November 5, 1996.

This new drug application provides for the following indication: Aricept™is indicated for the treatment of mild to moderate dementia of the Alzheimer's Type.

We have completed the review of this application, including the enclosed draft labeling, and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the enclosed draft labeling. Accordingly, the application is approved effective on the date of this letter.

The final printed labeling (FPL) must be identical to the enclosed draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit sixteen copies of the FPL as soon as it is available, in no case more than 30 days after it is printed. Please individually mount ten of the copies on heavy weight paper or similar material. For administrative purposes this submission should be designated "FINAL PRINTED LABELING" for approved NDA 20-690. Approval of this submission by FDA is not required before the labeling is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

Phase IV Commitment:

We remind you of your Phase 4 commitment specified in your submission dated October 3, 1996 in which you commit

These reports should be submitted to your NDA as correspondence. For administrative purposes, all submissions, including labeling supplements, relating to this Phase 4 commitment must be clearly designated "Phase 4 Commitments."

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any problems that may be identified. We note that you have proposed an method for identification of the drug substance in the dosage forms. We will contact you regarding possible improvements in the method.

Please submit one market package of the drug when it is available.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact:

Katurah Higgins, R.Ph.
Project Manager
(301) 594-5504

Sincerely yours,

Robert Temple 4/25/96

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

ENCLOSURE

Higgins

NDA# 20-690

Eisai America, Inc.
ATTN: Nicholas J. Farina, Ph.D.
Glenpointe Centre East
300 Frank W. Burr Blvd.
Teaneck, New Jersey 07666-6741

MAY 21 1996

Dear Dr. Farina:

We have received your new drug application (NDA) submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for the following:

Name of Drug Product: Aricept™ (donepezil hydrochloride) Tablets

Therapeutic Classification: P

Date of Application: March 29, 1996

Date of Receipt: April 5, 1996

Our Reference Number: NDA 20-690

Unless we notify you within 60 days of the receipt date that the application is not sufficiently complete to permit a substantive review, this application will be filed under section 505(b) of the Act on June 5, 1996 in accordance with 21 CFR 314.101(a).

Under 21 CFR 314.102(c) of the new drug regulations and in accordance with the policy described in the Center for Drug Evaluation and Research Staff Manual Guide CDER 4820.6, you may request an informal conference with this Division (to be held approximately 90 days from the above receipt date) for a brief report on the status of the review but not on the application's ultimate approvability. Please request the meeting at least 15 days in advance. Alternatively, you may choose to receive such a report by telephone. Should you wish a conference, a telephone report, or if you have any questions concerning this NDA, please contact:

Katurah Higgins
Project Manager
(301)594-5529

Please cite the NDA number listed above at the top of the first page of any communications concerning this application.

Sincerely yours,

5/21/96

John S. Purvis
Chief, Project Management Staff
Division of Neuropharmacological Drug Product
Office of Drug Evaluation I
Center of Drug Evaluation and Research

APPROVABLE

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-690

SEP 18 1996

Eisai America, Inc.
Attention: Nicholas J. Farina, Ph D.
Glenpointe Centre East
300 Frank W. Burr
Teaneck, NJ 07666-6741

Dear Dr. Farina:

Please refer to your March 29, 1996 new drug application submitted under section 505(b) of the Federal Food, Drug and Cosmetic Act for Aricept™ (donepezil HCl) 5 mg and 10 mg Tablets.

We acknowledge receipt of your submissions dated:

04-05-96	06-03-96	06-27-96	07-09-96	08-02-96
05-01-96	06-04-96	06-28-96	07-15-96	09-04-96
05-02-96	06-05-96	07-02-96	07-18-96	
05-13-96	06-11-96	07-03-96	08-01-96	

We have completed the review of this application as submitted with draft labeling, and it is approvable. Before this application may be approved, however, it will be necessary for you to respond to the following issues and adopt as labeling for Aricept™, the draft package insert attached to this letter, modified as requested (i.e., as per this letter and the notes embedded within the text of the attached package insert).

Package Insert

The attachment to this letter provides a draft of the labeling that the Agency proposes be adopted for Aricept™ Tablets upon its approval. Although sections of this proposal are taken verbatim from the labeling proposed by you in the NDA, other sections have been extensively revised. Please note that we have embedded throughout the text of the attached draft labeling, "Notes to Firm:", requesting further revisions or clarification of the label. We have the following specific comments and requests for the following sections of labeling.

1. CLINICAL PHARMACOLOGY: Clinical Trials subsection:

   Although there are many ways the data could be displayed, we would prefer that the description of the effects of donepezil in clinical use be based on the observed cases data set as defined in the protocols for studies 301 and 302, i.e., patients completing the study, including all patients, irrespective of compliance or protocol violations. For this reason, we request that all graphics, tables and

numerical citations be modified to use this data set rather than the intent to treat data set. The values provided should be unadjusted (i.e., not least mean square estimates). In the event that additional analysos are required to generate results, we ask that you provide us with the data and analytical models used.

We also note that you did not include data for the withdrawal phase of the two studies in Figures 1 and 4. This is a critical oversight; the prescriber needs to know about the consequences of treatment withdrawal, and the result of withdrawal has mechanistic implications. Our text does not include any statement regarding the pace at which the effects of Aricept™ are lost in view of the fact that you have not studied this issue systematically (only one observation is made following withdrawal and that occurs at the end of the withdrawal period).

We have eliminated the two illustrations of the CIBIC-plus results over time provided in the Clinical Trials subsection for studies 301 and 302. In our view, the CIBIC-plus, like many global assessments which have no landmarks and are highly study and assessor dependent, conveys minimal clinically useful information beyond the conclusion that an effect was detected in the clinical trial.

We have also eliminated statements that report the treatment effect in terms of improvement per week in a manner that implies it increases linearly and monotonically over the course of treatment. The analyses of the studies do not support this, a point illustrated by the plots of the ADAS-cog over time.

Finally, our description of the trials eliminates any reference to "treatment successes" as this term uses a definition (no worsening) that is an arbitrary criterion for a "success". The figures showing response distribution should allow physicians to understand the array of responses seen.

2. CLINICAL PHARMACOLOGY: Pharmacokinetics subsection: &
PRECAUTIONS: Drug Interactions subsection

Although the pharmacokinetics of Aricept are, in many respects unremarkable (e.g., linear, not dose dependent, not time dependent), the fact that it is metabolized by CYP 3A4 and CYP 2D6 requires certain additions to labeling and further study of potential interactions and individual differences. In vitro data indicate that Aricept is unlikely to interfere with the metabolism of drugs metabolized by CYP450, 2D6 or 3A4 but suggest that inhibitors of CYP450,2D6, and 3A4 could affect clearance. This should be studied and we would like your commitment to do so.

Also, because CYP 2D6 displays a genetic polymorphism (8% of Caucasians are classified as poor metabolizers), you should ascertain whether people deficient in this enzyme have an impaired ability to metabolize donepezil and significantly altered clearance.

Finally, we suggest studies to investigate the potential of enzyme induction by Aricept™.

3. ADVERSE REACTION section:

At this time, the number of patients for which we have complete safety information is limited to studies 201, 202, 301 and 302; in toto, this represents experience gained in no more than 700 to 800 subjects. Moreover, experience at the highest recommended dose, especially in extended use, is exceedingly limited (approximately 100 patients at 6 months). The numbers and rates presented in labeling must be updated to include information provided in the safety update. See Safety Update section below.

If additional information relating to the safety or effectiveness of this drug becomes available, revision of this labeling proposal may be required.

Chemistry, Manufacturing and Controls

1. We note that the drug product made at and at Pfizer/Brooklyn use different film coatings. Please provide an explanation for this. We request that you standardize on a single coating composition for each strength. Please also describe the Identity test you perform for the colorant in the

2. The Identification test(s) for the drug product are not acceptable. We request that you develop, provide and implement a spectrophotometric Identity procedure, specifically an for identification of the drug substance in the drug product. Should an test not be feasible, we request that you describe the attempts to develop such a method. In the event an method cannot be developed, Identity of the drug product will require the use of two distinct chromatographic procedures; i.e., one are not acceptable.

3. We note that you have concluded that drug product specifications for impurities and degradants are not necessary. While we concur that degradant levels are low in the stability studies conducted to date, these studies (on the product to be marketed) have been of minimal duration. We request that you incorporate a

general impurity/degradant limit into the drug product specifications. Such a limit may be that used with the Pfizer product [see Volume 14 page 14], or a more general specification, such as "Total Impurities no single impurity (either known or unidentified) greater than '.

4. We note that the Pfizer manufacturing instructions provide for rework of coated tablets. Such provision is not acceptable [i.e., should be withdrawn from the NDA] unless you can document the rework validation and demonstrate performance characteristics that meet specifications for the reworked tablets.

5. Please identify the supplier(s) of the starting material

6. Please provide a list of equipment used for the new drug synthesis which includes size and type/model as used for making commercial size batches of donepezil hydrochloride.

7. We note that the ranges in your synthesis description appear quite broad. Please provide a revised general synthesis description for donepezil hydrochloride which conforms to the ranges (e.g., time and temperature) which have been validated in actual manufacture.

Biopharmaceutics

We ask that the following final dissolution methodology and specification be adopted for Aricept 5 mg and 10 mg film-coated tablets:

Medium:
Apparatus:
Specification:

Although not requirements for approval, we have the following recommendations and requests for additional biopharmaceutic information (please note that recommendations involving *in vitro* drug-drug interaction studies have been discussed earlier):

1. We recommend that you repeat your *in vitro* metabolism studies using relevant therapeutic concentrations of Aricept to determine the specific isozyme responsible for the metabolism of Aricept. We note that the concentrations of Aricept used in the submitted *in vitro* studies were so high (μM) that switching from a high affinity, low capacity enzyme (e.g., CYP 2D6) to a low affinity, high capacity enzyme (e.g., CYP 3A4) is a clear possibility.

2. In your drug metabolism and elimination study, you have provided $AUC_{0-\infty}$ values for total radioactivity and E2020 in plasma; however, in order to determine the amount of exposure to metabolites, provide the individual $AUC_{0-\infty}$ values.

Phase 4 Commitment

We remind you of your commitment to complete (Phase 4). Please provide an estimate of when the reports of these studies will be submitted.

Safety Update

Under 21 CFR 314.50(d)(5)(vi)(*b*), we ask that you update your NDA by submitting all safety information you now have regarding your new drug. Please provide updated information including all deaths and any adverse events that led to discontinuation of the drug and any information suggesting a substantial difference in the rate of occurrence of common but less serious adverse events. The update should cover all studies and uses of the drug including: (1) those involving indications not being sought in the present submission, (2) other dosage forms, and (3) other dose levels, etc. Please include the following:

A table that enumerates the dose and duration of patient exposure to Aricept. This table should include the mean daily dose as well as the duration of administration. A table for each study as well as an overall exposure table should be provided in both paper and as an electronic data set.

Also include the following information:

1. Tables comparing adverse reactions at the time the NDA was submitted and for the updated safety data.

2. Tables of drop-outs with new drop-outs identified. Discuss, if appropriate.

3. Details of any significant changes or findings, if any.

4. A summary of worldwide experience on the safety of this drug.

5. Case report forms for each patient who died during a clinical study or who did not complete a study because of an adverse event.

6. Electronic data sets for the safety data domains for studies 202, 303, and 305.

Other

Also, please submit three copies of the introductory promotional material that you propose to use for this product. All proposed materials should be submitted in draft or mock-up form, not final print. Please submit one copy to this Division and two copies of both the promotional material and the package insert directly to:

Food and Drug Administration
Division of Drug Marketing, Advertising and Communications,
HFD-40
5600 Fishers Lane
Rockville, Maryland 20857

Within 10 days after the date of this letter, you are required to amend the application, notify us of your intent to file an amendment, or follow one of your other options under 21 CFR 314.110. In the absence of such action FDA may take action to withdraw the application.

The drug may not be legally marketed until you have been notified in writing that the application is approved.

Should you have any questions, please contact: Katurah Higgins, R.Ph.
Project Manager
Telephone: (301) 594-5504

Sincerely yours,

Robert Temple, M.D.
Director
Office of Drug Evaluation I
Center for Drug Evaluation and Research

Attachment(1)

DRUG STUDIES IN PEDIATRIC PATIENTS
(To be completed for all NME's recommended for approval)

NDA # 20-690 Trade (generic) names Aricept (donepezil Hcl) Tablets

Check any of the following that apply and explain, as necessary, on the next page:

____ 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.

____ 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(c) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.

____ a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.

____ b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate.)

____ 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children).

____ a. The applicant has committed to doing such studies as will be required.

____ (1) Studies are ongoing.
____ (2) Protocols have been submitted and approved.
____ (3) Protocols have been submitted and are under review.
____ (4) If no protocol has been submitted, on the next page explain the status of discussions.

____ b. If the sponsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request.

X 4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

____ 5. If none of the above apply, explain.

Explain, as necessary, the foregoing items: _____

Katurah N. Higgins — 8/7/96
Signature of Preparer — Date

cc: Orig NDA
HFD-120/Div File
NDA Action Package

DEBARMENT CERTIFICATION

On behalf of Eisai America, Inc., I hereby certify that we did not and will not use any capacity the services of any individual, partnership, corporation, or association debarred under subsections (a) or (b) of Section 306 of the Federal Food, Drug and Cosmetic Act in connection with NDA 20-690 for ARICEPT™ (donepezil hydrochloride).

Sharon L. Rogers, Ph.D.
Eisai America, Inc.
Vice President
Clinical Research

Memorandum **Department of Health and Human Services**
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

DATE: November 22, 1996

FROM: Paul Leber, M.D.
Director,
Division of Neuropharmacological Drug Products
HFD-120

SUBJECT: Approval of NDA 20-690, Aricept™ (donepezil)

TO: File NDA 20-690,
&
Robert Temple, M.D.
Director, Office of New Drug Evaluation 1

This memorandum conveys my conclusion that the **NDA for Aricept™ (donepezil)**, submitted by Eisai America, Inc. on **3/29/96**, and declared **approvable** on **September 19, 1996**, may be approved.

The basis for my conclusion that Aricept has, within the meaning of the Act, been shown to be effective in use and safe for use is provided in my 8/30/96 memorandum to the file.

Beyond the adequacy of the safety update, the primary substantive issue affecting my view of the application's final approval is product labeling[1].

The sponsor initially offered labeling that differed substantially from the draft provided in the approvable action letter, labeling upon which we had conditioned final approval of Aricept. Generally, the sponsor sought labeling that in my view was potentially false and misleading in that it could be used to imply the existence of advantages and distinctions between Aricept and the other drug product currently marketed for use as an antidementia agent (i.e., Cognex aka tacrine) that had not been evaluated, let alone documented. For example, the firm sought to represent their idiosyncratic CIBIC plus assessment in a manner

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[1] There are also some CMC issues that must be communicated, but none of these must be resolved prior to the approval of the NDA.

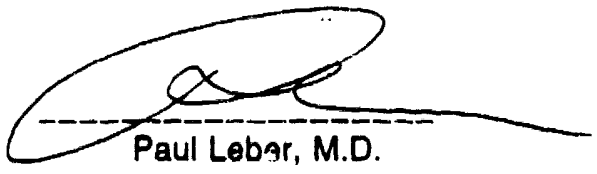
suggesting that it not only had a numerical quantitative interpretation with public meaning, a property that global assessments simply do not possess, but in a manner that might have allowed an inference that the numerical changes on their global could not only be compared to, but were superior to, the CIBI scores obtained with Cognex. We persuaded the sponsor that such a use of their CIBIC plus data was inappropriate.

Thus, through a process of iterative negotiation, a version of labeling text was developed that is acceptable to both the Division and the sponsor. Accordingly, I am now able to conclude that, within the meaning of the Act, Aricept is safe for use and effective in use as proposed for use in the labeling that is being forwarded as an attachment to the approval action letter.

I do wish to note for the record, however, that the warrant of safety is based on a smaller number of patients (reports of safe passage derive from experience with fewer than 1000 US patients; of these less than 600 have been exposed to 10 mg a day for 90 or more days) than we had anticipated would be available at the time the approvable action was taken. Although this shortfall does not affect my conclusion about the application's suitability for approval, it does lead me to reiterate the caveat, offered in my approvable action memorandum, that the extent of experience with Aricept limits the strength of the warrant that can be offered vis a vis Aricept's freedom from risks not observed. As a consequence, no person should be surprised if adverse clinical events, some quite serious, either not seen and/or not fully appreciated during Aricept's development, are reported post-marketing.

Conclusion:

The application may be approved.



Paul Leber, M.D.
11/22/96

Memorandum Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research

DATE: August 30, 1996

FROM: Paul Leber, M.D.
Director,
Division of Neuropharmacological Drug Products
HFD-120

SUBJECT: NDA 20-690, Aricept™ (donepezil)

TO: File NDA 20-690,
&
Robert Temple, M.D.
Director, Office of New Drug Evaluation 1

This memorandum conveys my endorsement of the review team's unanimous recommendation that the **NDA for Aricept™ (donepezil)**, submitted by Eisai America, Inc. on **3/29/96**, be **declared approvable.**

Introduction

The reports submitted to NDA 20-690 contain sufficient information to establish, within the meaning of the Act, that Aricept™ (donepezil) will be "effective in use" and "safe for use" if marketed under the conditions of use described in the version of draft labeling[1] developed by the Division.

In the course of the Division's systematic review of the information and reports provided in the NDA, no finding or issue has been uncovered that can reasonably be considered exceptional, disconcerting, or controversial. Accordingly, the NDA has not been presented to the Peripheral and Central Nervous System Drug Products Advisory Committee.

A caveat is in order, however. Although there is sufficient information to justify an approvable action at this time, it would be imprudent not to take note of the relatively modest size of the clinical safety data base upon which our conclusions are based. A more extensive discussion of the limitations imposed by the size of the data base is provided in a later section of this memorandum.

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[1] attachment to the draft approvable action letter being forwarded, in the company of this memorandum, to the Director of ODE 1 for signature.

NDA 20-690 was designated, at the instruction of the Director of ODE 1, as a **"P" application.** This decision reflects his appreciation of the advantage offered by an antidementia drug that does not require patients using it to undergo weekly blood tests to monitor for potential serum transaminase elevations. In other respects, however, Aricept would appear to offer no advantage to Cognex. (There is no head to head clinical comparison, however, of the two drug products in a valid clinical experiment).

As a consequence of its "P" designation, the **"quasi-PDUFA date for the NDA is 10/04/96** (the official date, however, remains 4/4/97).

Effectiveness in Use.

The sponsor has submitted reports of two [2] clinical investigations, **Study 301** and **Study 302**, that provide results that satisfy what are tantamount to the agency's current requirements for substantial evidence of effectiveness for an antidementia drug product.

Outcome in each trial was evaluated, as per our current guidance, using a dual outcome assessment strategy. The ADAS cog, a performance based, multi-item measure of cognition, served to document the domain of action of the product, and a proprietary[3] variant of the CIBIC-plus was employed to gauge

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[2] There is, in fact, a third adequate and well controlled trial conducted by the sponsor, Study 201, that failed to provide results that qualify it as a source contributing to the body of evidence that would, under current policy, be deemed substantial for an antidementia claim. Nothing in Study's 201's outcome undermines the conclusion that E2020 is effective, however. In fact, the change in the ADAScog score for its 5 mg/day group, but not its 3 or 1 mg groups was statistically significant from that of placebo. Study 201's failure to attain statistical significance may be reasonably explained by its lower dose range (1,3, and 5 mg) and it low statistical power: 40 subjects per treatment group, rather than 150 or so in studies 301 and 302. Study 201 did, however, employ a clinician's global measure of change (CGIC), and that difference (e.g., a possible differential sensitivity between the CGIC and the CIBIC-plus) might also have contributed (vide infra).

[3] The version of the CIBIC-plus used in these studies must be viewed as proprietary in that it is a version that was used in the E2020 trials by Eisai, and, as far as it is known, in no other drug development program.

the global importance of the treatment from a clinical perspective. For both instruments, higher values denote greater impairment or severity. The ADAS cog score for a patient may range from 0 to 70, a typical normal elderly individual scoring 6 or less. The CIBIC plus is a 7 point, symmetrical categorical change measure (4 = no change, 7 maximum worsening, 1 maximum improvement). A note of caution is in order regarding the latter's use, however.

Although the ADAS cog is, to the extent we can tell, the same instrument that has been used in other clinical trials of anti-dementia drug products, the CIBIC-plus is not[4]. Accordingly, the effect size reported for the CIBIC-plus

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Accordingly, although it is scored in the same way as a CIBIC or CIBIC-plus used in other trials, these scores have no necessary correspondence with those reported in those other studies. This, admittedly, will also be true of CIBICs used by different companies.

[4] There is not much we can easily do about the situation, however, as the agency is on record as an institution as having accepted the recommendations made by several of its expert advisors at the 2nd Symposium on Antidementia Drug Development Guidelines concerning the fungibility of the CIBIC-plus and CIBIC. Those who pressed us to adopt this policy presumed that the CIBIC plus had greater face validity, reflecting actual clinical practice more than the CIBIC. I have always viewed this argument as irrelevant. The CIBIC-plus undoubtedly does correspond more closely to the way in which physicians in clinical practice follow their patients , but that is hardly a reason favoring the CIBIC-plus' adoption as an outcome assessment instrument for clinical research. In any event, for a number of reasons, some more political than scientific, that is what happened. As a consequence, a CIBIC plus, rather than a CIBIC has become the de facto standard for assessing the clinical relevance of the effects associated with the cognitive improvements detected by the ADAS cog. While the CIBIC-plus is, in theory, more likely to be sensitive to change than the CIBIC (no doubt a reason contributing to its popularity among commercial sponsors) because it makes use of information obtained from family and caregivers, I believe, personally, that the advantage is illusory. First, the CIBIC plus does not secure the kind of information that I sought to obtain when I first proposed to use a clinician's interview based global assessment as a means of evaluating the clinical relevance of an agent that produced a detectable change on some measure of cognitive performance. The source of information driving a CIBIC plus may still in part be a reflection of an expert clinician's interview based assessment but it is just as likely to be a score dominated by family or

in Eisai America's clinical trials cannot be compared meaningfully with that reported for the CIBIC-plus used in clinical trials conducted by other sponsors and investigators. Obviously, the same warning applies to comparisons of CIBIC-plus and CIBIC scores; although both are 7 point categorical assessments, they do not measure the same thing. In particular, the CIBIC scores obtained in pivotal 6 month long Cognex trial (e.g., 970-61) and the CIBIC-plus scores reported for E2020 in Study 302 cannot be compared. These observations have relevance in regard to the description of clinical trials results provided in Aricept™ product labeling (e.g., see approvable action letter).

Study 301 was a 12 week long clinical investigation of 461 Alzheimer's Disease patients (baseline ADAS cog ~ 26) who were randomized to placebo (N=150), 5 mg/day of E2020 (N= 156) or 10 mg a day of E2020 (N= 155).

On the **ADAS cog,** the differences among the treatments, and between each dose of E2020 and placebo were highly significant ($p \leq 0.0001$) for the intent to treat study sample. The adjusted least square means effect size on the ADAScog (drug vs placebo group difference in the extent of change from baseline) was 2.7 for the 5 mg dose and 3.2 for the 10 mg dose at 12 weeks. Of interest, the cognitive performance of patients in both drug groups, in contrast to those randomized to placebo, actually improved on average (i.e., change from baseline was -2.2 and -2.8 ADAS cog units for the 5 and 10 mg dose groups, respectively) at the end of the 12 week study.

On **the CIBIC-plus**, the differences among treatments and both pairwise drug placebo differences were again highly significant (all $p \leq 0.01$). There was no indication of a dose effect, however, although, the 5 mg/day group did ever so slightly worse numerically than the 10 mg/day group (3.9 vs. 3.85).

Study 302 was identical, save for its longer duration (24 weeks vs 12

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care-giver derived reports. It is an assumption, and a highly arguable one, that family members can assess the performance of patient more reliably than a clinician at an interview (stories told 3rd hand by untrained observers are always of questionable validity/reliability). Even worse, in my view, the input from families and/or caregivers increases the probability that the clinician assessor will become aware of the patient's treatment assignment, a result that totally undermines the validity of a global assessment.

weeks) in design to Study 301, comparing 5 and 10 mg doses of E2020 with placebo in a balanced randomization of some 473 SDAT patients to placebo: N=162, 5 mg E2020: N=157 and 10 mg E2020: N=157.

On the **ADAS cog,** the differences among the treatments, and between each dose of E2020 and placebo were highly significant ($p \leq 0.0001$) for the intent to treat study sample. The adjusted least square means effect size on the ADAScog (drug vs placebo group difference in the extent of change from baseline) was 2.8 among 5 mg assigned subjects and 3.2 units among those on the 10 mg dose at week 24. Over this longer interval, surprisingly, both treatment groups, were on average, better than they were at baseline. (mean ADAS cog change from baseline for the 5 and 10 mg groups was -0.9 and -1.32 units respectively.)

On **the CIBIC-plus**, the differences among treatments and both pairwise drug placebo differences were again highly significant (all $p \leq 0.01$). There was a suggestion of a dose effect, however. Not unexpectedly, given the length of the trial, the mean scores of all groups indicated worsening, albeit slight in all 3 groups: 4.5, 4.2 and 4, respectively for the placebo, 5 mg and 10 mg groups. The direction of the change in the CIBIC plus, therefore, is inconsistent with that observed on the ADAS cog.

Comment: The lack of deterioration in cognitive performance (at least as measured by the direction of changes from baseline in the mean group ADAS cog scores) among patients treated with E2020 might lead some to opine that it is hard to imagine that E2020 does not exert some kind of a neuroprotective effect. At least 2 findings suggest that this sanguine conjecture is unwarranted.

First, the Alzheimer's Disease patients who participated in the E2020 development program did unexpectedly well in the absence of treatment. In study 301, the placebo group deteriorated at an annualized rate of only 2 points per year (0.5 x 52/12); in Study 302, the rate was higher, about 4 points per year (1.9 x 52/24). These observed rates must be considered in light of the fact that longitudinal cohorts of SDAT patients similar to those enrolled in the two E2020 trials are expected to experience about an 8 to 12 point average annual increase in their ADAS cog scores.

An even more persuasive reason to discount an effect on the neuro-anatomical progression of Alzheimer's Disease as an explanation for the seemingly sustained nature of E2020's effects is the almost immediate

deterioration in cognitive performance that is observed following the withdrawal of E2020 at each study's end. In each study, the patients withdrawn from E2020 suffer a decrement in cognitive performance (measured after 3 weeks in Study 301 and 6 weeks in Study 302). In Study 302, the loss of gain is so great that at the end of that study's 6 week long washout period, the mean ADAScog scores for patients initially randomized to each E2020 group are indistinguishable from those randomized to placebo (Figure 1 b of Dr. Hoberman's review (6/20/96) illustrates this point graphically).

In sum, the evidence developed in 2 adequate and well controlled investigations is entirely consistent with a conclusion that E2020 exerts an effect on cognitive performance in Alzheimer's patients, an effect that is of sufficient size to be detected using a CIBIC plus. Under current policy, these results constitute substantial evidence of an antidementia treatment effect.

Safety in use

Preclinical safety

Although there are no findings among the reports of preclinical toxicological tests submitted that raise concerns about the potential of this reversible cholinesterase inhibitor to cause injury to humans[5], it bears note that results of in vivo life-time rodent carcinogenicity testing are not yet available. The absence of reports for such studies in the NDA is a consequence of a decision, initially taken during the course of tacrine's development, to adopt a generic policy allowing sponsors developing treatments for dementia to submit reports of carcinogenicity studies in phase 4. I note for the record that E2020 is clastogenic (but only at cytotoxic doses) in a chinese hamster lung cell test, but is not mutagenic in the Ames or mouse micronucleus tests.

Clinical Safety

General observations about the clinical safety data.

The information bearing on Aricept's safety is described in Dr. Levin's review

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[5] A conclusion of Dr. Rosloff that is endorsed by Dr. Fitzgerald, the team leader for Pharmacology (8/7/96)

of 7/1/96, his "team leader" memorandum of 8/2/96, and a 2 page addendum (8/27/96) that I asked him to prepare that enumerates the extent of exposure of individuals to Aricept by both time[6] and dose.

The determination that E2020 is "safe for use" derives from information, observation, and lab (and special) test results obtained from a number of different clinical sources. The quality of the evidence is not uniform, however, and varies with the kind of information reported upon.

Deaths, serious adverse events and events causing premature withdrawals from clinical trials are presented for all patients at risk, including those exposed in Japan during phase 1 and 2 to doses below 5 mg a day, the minimum dose that will be recommended upon marketing.

What we currently believe we know about the probable risks of E2020 at the doses proposed for use comes, therefore, from a relatively limited experience gained in approximately 600 to 700 patients who were exposed to doses of 5 or 10 mg a day for periods of 12 weeks or longer. I am mindful that for most routine indications, this number might well not be deemed sufficient to support an approvable action.

Given the nature of the dementia, the lack of fully satisfactory treatments available for its management, the kind of adverse events reported so far (cholinergic ADRs), and the clear cut documentation of E2020's effectiveness, it seems reasonable to make this application an exception to ordinary practice. This comes at a price, however. It requires that we tolerate uncertainty about risks associated with Aricept's use that occur at an incidence as high as 1 in a 100 or so, an incidence that is relatively high when considered from a public health perspective.

Fortunately, prior to any final approval action, the actual number of patients on whom we will have information is likely to increase substantially. Specifically, with the submission of the safety update that is required as a final condition of approval of the NDA, there may be reports on as many as 500 to 600 additional patients from both ongoing trails and from extensions to completed trials. Among these will be patients who were initially

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[6] Unfortunately, the exact time on dose for patients in the Japanese studies is not available. Accordingly, the estimates provided are based on planned exposures and are, therefore, likely to be too generous (see page 2, R. Levin, 8/27/96)

assigned to placebo and who accepted the opportunity, at the completion of their participation in the study, to take Aricept™ under open conditions. At the moment, however, the extent of exposure to 10 mg of Aricept, the highest dose that will be recommended in labeling is limited.

In his 7/1/96 safety review, Dr. Levin focuses on the information generated on some 747 E2020 exposed patients who participated in one of 3 completed RCTs (201, 301 and 302) and one open label extension trial (202).

I take note of the following:

Deaths:

There were 27 deaths that occurred on or within 4 weeks of exposure to E2020 among the patients reported in the NDA (the 3 controlled trials and the Japanese studies). Dr. Levin finds no basis to attribute E2020 a role in any of them; after reading his synopses of the cases, I see no reason to dispute his conclusion.

Discontinuations:

Taking the 3 American controlled trials (201, 301, and 302) as a pool, the incidence of discontinuation attributed to adverse clinical events among patients randomized to 5 mg a day of E2020 is 5 %, the same as that among placebo patients. The incidence is 13% among those randomized to 10 mg/day. Because I have not been provided with a tabulation of the incidence by dose, by week of discontinuations, I am unable to evaluate the time course of the dropouts. Accordingly, the firm is being asked to do so because this may provide some insight into what extent the change in dose rather than the dose itself contributes to this rate.

I do note that several cases of syncope were reported. All could, of course, have nothing to do with E2020, but I am sensitive to the fact that excess cholinergic vagal activity can slow, even pause the heart.

Serious Clinical Events

About 6% of the 747 patients in the safety database experienced a "serious" Adverse Event. The tabulation of these events provided in Dr. Levin's review shows no signal that the incidence is dose related, but the number of events is probably far too small to exclude the possibility.

Treatment Emergent Adverse Events

The tabulation provided by Dr. Levin documents that nausea and vomiting are common; they are, of course, expected consequences of the cholinomimetic action of E2020. Less commonly, but also seemingly linked to the use of E2020 (by intraocular test), are muscle cramps, fatigue, vomiting, anorexia and perhaps weight loss (10 mg /day, only).

Treatment Emergent Abnormal Lab Events

There are no blood chemistry or EKG's findings that would signal a risk specific to the use of E2020.

Biopharmacokinetics and Dose

PK-PD Issues

E2020 has a long half life of elimination (about 70 hours). At least 1 of E2020's metabolites, 6-O-desmethyl donepezil is active as a cholinesterase inhibitor and is present in substantive amounts in the plasma (circa 20% of the radioactivity after a single dose). Accordingly, maximal effects of E2020 may not be attained until a patient is maintained for 2 weeks or more at a given dose (i.e., is in steady state). Moreover, even if the postulated link between E2020's cholinesterase inhibitor activity and its therapeutic effects exists, it may not be direct. Accordingly, the latency to maximum effect of a given dose may be even longer than that predicted by the drug's elimination half-life.

These facts are not only conveyed in labeling, but influence what labeling text can reasonably state. For example, given the uncertainty of the linkage between RBC cholinesterase activity and the drug's therapeutic effects I see no basis for including the Emax model linking RBC inhibition and plasma concentration in product labeling.

The information on pharmacokinetics also affects dosing recommendations as I note in the following section.

Dosing Recommendations.

The firm's development of E2020, at least in the phase 3 American studies has focused on 5 and 10 mg a day doses. Lower doses (1, 3 and 5 mg were

used in study 201) and in uncontrolled Japanese studies, and the sponsor attests these, in aggregate, provide no results bearing on the product's effectiveness in use. Although it is tempting to view Study 201 as providing evidence of the lack of effectiveness of doses of 1 mg and 3 mg/day, it would be stretching a bit given the study's lack of power to document the effects of the 5 mg dose of E2020 on the CGIC.

The problem confronting us at this juncture is that based on the findings of the clinical investigations upon which we rely, there are no formal findings (statistically significant differences) that would justifying recommending a dose in excess of 5 mg a day. On the other hand, the data, by inspection, suggest that a numerically larger effect is seen in both studies 301 and 302 with the 10 mg dose. It is possible to interpret this as no more than a failure to conduct studies with sufficient power to prove the dose dependent differences are real.

I am reluctant to do so, however. Accordingly, I am proposing a narrative discussion of the problem in the Dosage and Administration section of labeling that draws attention to the fact that there is a suggestion in the evidence, but not proof, that a higher dose may be more effective. This section also takes note of the futility of trying to 'titrate' dose by therapeutic response with a drug of this kind in this population.

Metabolic route of elimination.

Aricept is metabolized by cytochrome P450 oxidative enzymes including both 2D6 and 3A4. The firm argues that the effects of Aricept on the metabolism of other drugs that are eliminated by these enzymes, in particular those with narrow therapeutic ratios is not likely to be clinically important because the maximal plasma concentrations likely to be attained with Aricept at 10 mg/day will be very low relative to the Ki for both these enzyme systems (based on in vitro experiments. The argument is logical enough, but in the absence of clinical trials, little more. Accordingly, the draft of labeling we have produced in the Division calls attention to this limitation.

The firm unfortunately has not even conducted in vitro experiments to determine to what extent drugs that are known inhibitors of CYP450 2D6 and 3A4 systems will affect the metabolism of Aricept. They fail to acknowledge this fact in their proposed labeling. The division's draft labeling makes prominent note of this limitation in a bolded advisory statement; removal of this advisory statement is expressly linked to the

firm's conducting of tests evaluating the potential of common drugs, known 2D6 or 3A4 inhibitors, to interfere with the clearance of Aricept™.

Other Labeling Issues

A number of other issues in regard to labeling are taken up in the text of the letter to the firm and/or the body of the labeling itself. I will not review these matters. Suffice it to say that our interventions are intended to prevent the firm from overstating the strength of the evidence bearing on efficacy and safety of Aricept.

Chemistry

Inspections of manufacturing facilities in Japan are not planned until mid to late September. The ONDC is aware of the short time between the inspection date and the PDUFA date.

DSI

Routine Inspections.

At least one routine inspection has not yet been completed.

Unsubstantiated allegation concerning the integrity of the evidence upon which the sponsor relies.

A xeroxed copy of a one page letter, dated August 1, 1996, without signature, but identified as having been written by "Concerned Investigators," was sent to me through the mails. It alleges that "a senior official at Quintiles, Kevin Keim Ph.D., personally visited a number of investigator sites during the conduct of this [?] study, and instructed investigators to change their scores of the CIBIC-plus evaluation." The letter implies that it is directed at the Phase II (3 month) and Phase III (6 month trials)

I sent the copy of the letter and envelope I received to DSI on 8/15/96.

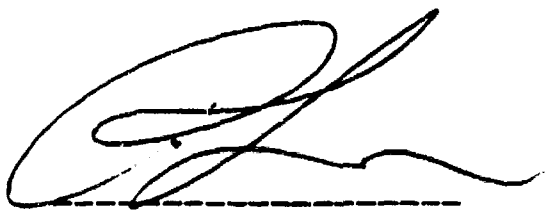
Summary and Conclusions:

The reports provided in NDA 20-690 support a conclusion that, within the

meaning of the Act, Aricept will be safe for use and effective in use provided it is 1) distributed for use under the conditions described in product labeling and 2) that the firm satisfactorily complies with the requests conveyed in the approvable action letter being forwarded to the Office of New Drug Evaluation 1.

In reaching this conclusion I am mindful of the limited amount of information upon which our inferences about the safety of Aricept are based. I am persuaded, however, that if effective drugs are to be rapidly developed and expeditiously evaluated for the treatment of serious illnesses such as Alzheimer's Dementia, this is the price society must pay. Society , in turn, must accept the fact that Aricept may well cause injury and harm at a high incidence of a kind of which we have yet to see even a single example. Indeed, society will also have to live with the possibility that events that have been reported and noted in the NDA database occur at an increased incidence in association with Aricept's use (e.g., syncope, pancreatitis, etc.). Unfortunately, we simply can't know at this time whether or not these events are or are not causally linked to Aricept™.

This caveat about our uncertainties offered, I recommend that the approvable action letter be issued.



Paul Leber, M.D.
8/29/96, 4:20 pm

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neuropharmacological Drug Products (HFD-120)
Center for Drug Evaluation and Research

Date: 8/2/96

From: Randy Levin, M.D., Neurology Team Leader
Division of Neuropharmacological Drug Products, HFD-120

Subject: NDA 20-690, Aricept (donepezil, e2020)

To: file

Introduction:

Aricept (donepezil, e2020) is a reversible acetylcholinesterase inhibitor (AChEI). It is a piperidine based inhibitor compared to tacrine which is a acridine based molecule.

Eisai submitted IN , for the evaluation of e2020 in the treatment of Alzheimer's disease (AD) on 12/20/90 and the NDA was submitted on 3/29/96.

I reviewed the safety and efficacy information provided in the NDA. Other portions of the NDA were reviewed by Dr. Rzesotarski (chemistry), Dr. Rosloff (pharm/tox), Dr. Tammara (PK) and Dr. Hoberman (statistics).

Overview of the clinical program:

Phase 1:

Eighteen Phase 1 studies were conducted in Japan, UK and US and included PK studies, drug interaction studies and studies in special populations. A total of 285 subjects were enrolled with 262 receiving drug for up to 5 weeks. In phase 1 studies, the drug was found to be readily absorbed without significant first pass metabolism. 95% is bound to human plasma proteins. The terminal half life was about 70 hours with steady state achieved in 15 days. The PK is linear from doses of 2 mg to 10 mg.

Phase 2:

Seven phase 2 studies were conducted in Japan. The study reports were not

included in the NDA but serious AEs were reported in the integrated summary of safety. The safety results were reported by the sponsor to be similar to those seen in the US population. GI adverse events were most common.

One phase 2 study, 201, was conducted in the US. This was a 12 week, randomized, double blind, placebo controlled, parallel, study evaluating doses of 1, 3 and 5 mg/day. Patients completing this study were eligible to be enrolled into study 202, an open label extension. Following a series of protocol amendments, patients were allowed to be titrated to a maximum of 10 mg/day.

Phase 3:

Two phase 3 studies were conducted in the US, studies 301 and 302. Study 301 and 302 had similar designs. These studies were randomized, placebo controlled, double blind, parallel trials evaluating doses of 5 and 10 mg/day. In study 301, the double blind phase of the study was 12 weeks while in study 302, the duration of the double blind phase was 24 weeks. Patients were eligible for a long term extension study, 303. A study report for study 303 was not provided in the NDA. Deaths and serious AEs leading to withdrawal and lab values collected in study 303 prior to the cut off date are provided in the integrated summary of safety.

A third phase 3 study, 304, was ongoing as of the time of the NDA submission. It was being conducted outside the US and had a design similar to study 302 with doses of 5 and 10 mg/day being compared to placebo. 750 patients were to be enroll for this 24 week study. Patients were eligible for a long term extension, study 305. As of 12/31/95, 438 patients were enrolled in study 304. A study report was not provided with the NDA but deaths, serious AEs leading to withdrawal and lab values collected prior to the NDA cut off date were provided in the integrated summary of safety.

Efficacy data:

Overview of the pivotal and supportive efficacy studies

The sponsor identified studies 301 and 302 as adequate and well controlled studies providing definitive evidence for the efficacy of e2020 in the treatment of AD. They identified study 201 as a supportive trial. A review of the protocols confirmed that studies 301 and 302 were, by design, adequate for providing definitive evidence for efficacy for the symptomatic treatment of AD.

A comparison of these three studies is summarized in the following table:

Comparison of protocols for studies 201,301 and 302			
Parameter	Study 201	Study 301	Study 302
Age of patients	55-85	≥50	≥50
Doses evaluated (mg/day)	0, 1, 3 and 5 mg/day	0, 5 and 10 mg/day	0, 5 and 10 mg/day
Patients per group	40/group	150 per group	150 per group
Duration of double blind phase	12 weeks	12 weeks	24 weeks
Duration of placebo washout	2 weeks	3 weeks	6 weeks
Primary efficacy outcome measures	ADAS-COG, CGIC	ADAS-COG, CIBI-C plus	ADAS-COG, CIBI-C plus
Secondary outcome measures	MMSE, QOL, ADL, CDR	MMSE, CDR, QOL	MMSE, CDR, QOL
Time points for efficacy assessments	0,1,3,6,9,12,14 weeks	0,1,3,6,9,12,15 weeks	0,6,12,18,24,30 weeks

The results of the efficacy studies are summarized in the following table:

Summary of efficacy results (treatment effect for the highest e2020 dose[1] vs placebo using LOCF data set[2])			
Outcome measure	Study 201	Study 301	Study 302
ADAS-COG	* (-3.19)	* (-3.07)	* (-2.88)
CIBIC plus	n/a	* (-0.34)	* (-0.44)
CGIC	+ (-0.11)	n/a	n/a
MMSE	+ (1.11)	* (1.82)	* (1.36)
CDR	+ (-0.21)	+ (-0.17)	* (-0.6)
ADL	+ (-4.61)	n/a	n/a
QOL	- (3.34)	n/a	- (-2.05)

[1]Highest dose for study 201 was 5 mg while 10 mg was the highest dose for study 301 and 302.
[2]LS means used for treatment differences in all cases except for the CIBIC and CGIC
*Statistically significant in favor of e2020 when compared with placebo
+ No statistical difference but numerical difference in favor of the drug
- No statistical difference but numerical difference in favor of placebo

Study 302:

Disposition and demographics:

68% of the patients on 10 mg/day and 80% on placebo completed the 24 week study. Baseline characteristics were similar between groups. The mean MMSE score was 19. Patient disposition is summarized in the following table:

Study 302: Disposition-number of patients at each visit (%)			
	Placebo (n=162)	5 mg (n=154)	10 mg (n=157)
week 6 (%)	146 (90)	144 (94)	137 (87)
week 12 (%)	139 (86)	139 (90)	126 (80)
week 18 (%)	138 (85)	135 (88)	113 (72)
week 24 (%)	130 (80)	131 (85)	106 (68)
Discontinued (%)	32 (20)	23 (15)	51 (32)
Discontinued for AEs (%)	11 (7)	9 (6)	26 (17)

Primary outcome measures:

For the ADAS-COG and CIBIC plus, the treatment differences for both the 5 and 10 mg/day group compared to placebo were statistically significant at week 12 through week 24. The effect was consistent across centers. The results were similar for all data sets analyzed. 6 weeks after discontinuation of the study treatment, the differences between groups were associated with a p value > 0.05. The results are summarized in the following tables:

Study 302: ADAS-COG change from baseline (± SE)			
	Placebo	5 mg/day	10 mg/day
Baseline	27.3 (0.34)	26.1 (0.35)	27.4 (0.31)
Observed cases			
week 6	-1.51 (0.39)	-1.86 (0.35)	-1.83 (0.45)
week 12	0.80 (0.42)	-1.25 (0.41)	-1.97 (0.50)
week 18	1.90 (0.51)	-0.90 (0.41)	-1.48 (0.56)
week 24	1.91 (0.52)	-0.78 (0.42)	-1.26 (0.57)
week 30	2.82 (0.53)	2.70 (0.57)	1.89 (0.53)
LOCF	1.91 (0.49)	-0.69 (0.40)	-1.40 (0.51)
ITT	1.90 (0.49)	-0.61 (0.41)	-1.07 (0.50)

Study 302: CIBIC plus score (± SE)			
Observed cases	Placebo	5 mg/day	10 mg/day
week 6	3.97 (0.066)	3.94 (0.065)	3.96 (0.069)
week 12	4.20 (0.075)	3.99 (0.066)	3.92 (0.085)
week 18	4.40 (0.083)	4.10 (0.082)	3.94 (0.093)
week 24	4.53 (0.084)	4.20 (0.093)	4.09 (0.099)
week 30	4.75 (0.087)	4.45 (0.097)	4.65 (0.086)
LOCF	4.50 (0.080)	4.18 (0.091)	4.07 (0.086)
ITT	4.52 (0.080)	4.18 (0.091)	4.07 (0.086)

Responders were defined for a post hoc description of the data as those with no change or better on the 24 week CIBIC and ADAS-COG. 38, 46 and 64% of patients on placebo, 5 and 10 mg, fulfilled these criteria, respectively.

Study 301:

Disposition and demographics:

83% of the patients on 10 mg/day and 93% on placebo completed the 12 week study. Baseline characteristics were similar between groups. The mean MMSE score was 19. Patient disposition is summarized in the following table:

Study 301: Disposition of patients

	Placebo	5 mg	10 mg
Number of patients at each visit			
Baseline	153	157	158
week 3 (%)	150 (98)	149 (95)	146 (92)
week 6 (%)	147 (96)	145 (92)	137 (87)
week 9 (%)	145 (95)	142 (90)	131 (83)
week 12 (%)	142 (93)	142 (90)	131 (83)
Discontinued (%)	11 (7)	16 (10)	29 (18)
Discontinued for AEs (%)	3 (2)	7 (4)	16 (10)

Primary outcome measures:

There was a statistically significant difference between the 5 and 10 mg/day group compared to placebo at all timepoints for the ADAS-COG and at 12 weeks for the CIBIC plus. 3 weeks after discontinuation of the treatment, there continued to be a statistically significant difference between groups. The results were consistent across groups and data sets analyzed. The results are summarized in the following tables:

Study 301: ADAS-COG change from baseline (±SE)			
	Placebo	5 mg/day	10 mg/day
Baseline (±SE)	25.7 (0.82)	26.2 (0.88)	26.6 (0.87)
Observed cases			
week 3	-0.69 (0.30)	-1.98 (0.36)	-3.03 (0.36)
week 6	-0.14 (0.35)	-1.55 (0.34)	-3.01 (0.45)
week 9	-0.27 (0.36)	-1.98 (0.39)	-2.52 (0.44)
week 12	0.45 (0.41)	-2.22 (0.45)	-2.68 (0.50)
week 15	1.5 (0.45)	-0.57 (0.41)	-1.61 (0.47)
LOCF	0.44 (0.39)	-2.12 (0.43)	-2.69 (0.47)
ITT	0.42 (0.39)	-2.17 (0.43)	-2.64 (0.47)

Study 302: CIBIC plus scores (± SE)			
	Placebo	5 mg/day	10 mg/day
Observed cases			
week 3	3.98 (0.052)	3.98 (0.053)	3.86 (0.062)
week 6	3.98 (0.066)	3.84 (0.068)	3.91 (0.068)
week 9	4.03 (0.076)	3.74 (0.082)	3.84 (0.068)
week 12	4.19 (0.074)	3.85 (0.081)	3.82 (0.082)
week 15	4.25 (0.080)	4.07 (0.088)	4.10 (0.088)
LOCF	4.20 (0.072)	3.87 (0.078)	3.82 (0.079)
ITT	4.21 (0.072)	3.87 (0.079)	3.82 (0.079)

Responders were defined for a post hoc description of the data as those with no change or better on the 12 week CIBIC and ADAS-COG. 40, 64 and 61% of patients on placebo, 5 and 10 mg, fulfilled these criteria, respectively.

Study 201:

Disposition and demographics:

92% of the patients on 10 mg/day and 90% on placebo completed the 12 week study. Baseline characteristics were similar between groups. Patient disposition is summarized in the following table:

Study 201: Disposition of patients				
	Placebo	1 mg	3 mg	5 mg
Number of patients at each visit				
Baseline	40	42	40	39
week 3 (%)	37 (93)	39 (93)	40 (100)	38 (97)
week 6 (%)	36 (90)	37 (88)	40 (100)	35 (90)
week 9 (%)	35 (88)	36 (86)	39 (98)	35 (90)
week 12 (%)	36 (90)	35 (83)	38 (95)	36 (92)
Discontinued (%)	5 (13)	8 (19)	2 (5)	5 (13)
Discontinued for AEs (%)	2 (5)	5 (12)	2 (5)	3 (8)

Primary outcome measure:

Patients on 5 mg/day had a statistically significant difference compared to patients on placebo for the ADAS-COG at week 3, 9 and 12. The differences between the week 12 CGIC while not statistically different were numerically in favor of the drug. The results are summarized in the following table:

Study 201: ADAS-COG change from baseline (±SE)				
	Placebo	1 mg/day	3 mg/day	5 mg/day
Baseline (±SE)	27.2 (1.78)	26.6 (1.52)	29.2 (1.71)	29.1 (2.00)
Observed cases				
week 3	-0.91 (0.77)	-1.82 (0.80)	-3.09 (0.66)	-3.77 (0.75)
week 6	-0.42 (0.79)	-1.92 (0.73)	-2.40 (0.66)	-2.30 (0.70)
week 9	0.01 (0.83)	-1.46 (0.71)	-1.35 (0.78)	-3.11 (0.69)
week 12	0.64 (0.82)	-1.12 (0.76)	-1.28 (0.76)	-2.52 (0.59)
week 14	1.37 (0.70)	1.10 (0.68)	0.41 (0.87)	-2.00 (0.71)
LOCF	0.66 (0.75)	-0.87 (0.68)	-1.33 (0.72)	-2.43 (0.54)

Study 201: CGIC scores (±SE)				
Observed cases	Placebo	1 mg/day	3 mg/day	5 mg/day
week 0	3.98 (0.025)	3.81 (0.13)	4.05 (0.05)	4.03 (0.045)
week 3	3.76 (0.098)	3.92 (0.093)	3.85 (0.098)	3.80 (0.080)
week 6	3.92 (0.14)	3.78 (0.088)	3.75 (0.10)	3.80 (0.11)
week 9	3.91 (0.14)	4.06 (0.13)	3.77 (0.10)	3.74 (0.10)
week 12	3.81 (0.15)	3.83 (0.12)	3.86 (0.11)	3.72 (0.12)
week 14	4.11 (0.16)	4.05 (0.11)	3.92 (0.12)	3.89 (0.11)
LOCF	3.85 (0.15)	3.88 (0.11)	3.90 (0.11)	3.74 (0.11)

Safety data:

Indication:

The sponsor is recommending 5 mg of e2020 per day as the initial dose. A dose increase to 10 mg/day can be considered after one month of treatment.

Source of safety data:

While the sponsor has conducted 32 studies, the majority of the safety

information on the drug comes from 3 placebo controlled studies; 201, 301 and 302. In studies 201 and 301, patients were exposed to the drug for up to 12 weeks and in study 302, 24 weeks. For long term safety, the sponsor has provided information from study 202, the long term extension of study 201. To support the safety of the drug, the sponsor has included information on serious AEs and withdrawals from the other studies. They have also included information about serious AEs and lab abnormalities from study 303, the long term extension for studies 301 and 302.

Exposure:

In phase 1 and 2 studies, the sponsor evaluated doses up to 5 mg/day. In the phase 3 studies, 5 and 10 mg/day doses were evaluated. A summary of the exposure for studies 201, 202, 301 and 302 is in the following table:

Number of patients exposed to e2020 by dose and duration (studies 201, 202, 301 and 302)								
All doses			5 mg			10 mg		
Any	> 3 months	> 6 months	Any	> 3 months	> 6 months	Any	> 3 months	> 6 months
787	625	337	450	383	223	382	240	107

Since the maximum dose in phase 2 was increased to 10 mg/day after the initiation of the study, the long term exposure to doses of 10 mg is limited. In study 202, only 50 patients had been exposed to doses of ≥ 5 mg/day for 1 year and no patients had been exposed to doses of 10 mg/day for ≥ 1 year.

Information about the safety of the 10 mg/day dose in durations over 6 months comes from study 303. In study 303, patients from study 301 and 302 were treated with doses of 5 or 10 mg/day. Information on serious AEs and lab values of over 700 patients enrolled into study 303 from study 301 and 302 were included in the safety data base though a formal study report was not included in the NDA. In study 303, as of the cut off date for the NDA, 196 patients were exposed to e2020 for ≥ 50 weeks. 95% of the patients in study 303 were on doses of 10 mg/day.

Deaths:

There were a total of 27 deaths that occurred within 4 weeks of treatment of

e2020 in all studies. There were 4 deaths out of the 1102 patients (0.4%) during the 3 to 6 month controlled clinical trials 201, 301 and 302. 3 of 355 (0.8%) placebo treated patients and 1 of 315 (0.3%) patients on 10 mg/day died during these studies. No patients on 1, 3 or 5 mg/day died while in the controlled clinical trials.

There were 17 deaths in the long term extension studies 202, 303 and 305. These trials involved over 900 patients as the cut off date of the NDA. 3 of 133 patients in study 202 died (2.2%) , 9 of 767 (1.2%) patients exposed in study 303 died. 4 patients in study 305 died. The total number of patients exposed in study 305 is not known but it is < 488 patients. The remaining deaths occurred in study 304, an ongoing blinded 24 week efficacy study. 5 of 488 (1.0%) patients exposed in study 304 died. One patient involved in a 190 (0.5%) patient Japanese study died.

The mortality rates in the long term studies are expected to be higher than in the controlled trials because the duration of involvement is longer. The mean number of days on drug at the time of death for the open label studies 202, 303 and 305 was 187 days compared to the 85 days in the controlled clinical trials 201, 301, 302 and 304.

The deaths were related to causes common in this age group including cardiac events (7 patients), pneumonia including aspiration pneumonia (5 patients), stroke (4 patients) and cancer (4 patients) (bladder, pancreatic, lung and metastatic). Other causes of deaths were pulmonary embolism (2 patients), pancreatitis associated with cholelithiasis (2 patients), accident (2 patients) and upper GI bleed (1 patient). Of the cases above, the following were associated with the use of placebo: cardiac, pulmonary embolism and upper GI bleed. The following cases are still blinded: pneumonia, cardiac (2 patients), pulmonary embolism and accident.

Discontinuations:

In the controlled clinical trials, over 75% of the patients enrolled completed the studies. 5% of the placebo patients, 5% of the patients on 5 mg/day and 13% of the patients on 10 mg/day discontinued for adverse events. Of patients entering study 202, the long term extension study, 76 of 133 (57%) discontinued. Most discontinued for lack of efficacy (21%) or by request (20%) rather than for AEs (11%).

Discontinuations for adverse events:

GI symptoms were the major cause of discontinuation for AEs in studies 201, 301 and 302. 20 of the 42 patients discontinuing for AEs had GI complaints. The majority of these complaints were for nausea, diarrhea and vomiting. Other AEs leading to withdrawal had incidence rates of ≤ 1%. The events that had an incidence of 1% in the 10 mg/day group when the rate in the placebo group was < 1% were accidents, fatigue, weight decrease, fractured bone, depression, aggression and insomnia. In study 303, seven patients withdrew with syncopal spells. This represents a rate of about 1% of the patients at risk. The duration of exposure in this population was not presented in the NDA. In study 202, 1 of 133 patients had a syncopal spell (0.75%)

Discontinuation for lab abnormalities:

Two patients withdrew for elevation of the ALT. One patient had an elevation to 163 on day 82 which returned to normal within 2 weeks of discontinuation of the drug. The other patient had an elevation to 508 on day 76. This also returned to normal levels within 3 weeks of discontinuation of the drug.

Other discontinuations:

Four patients discontinued for ECG abnormalities. The specific changes were supraventricular tachycardia, atrial fibrillation and multifocal PVCs. One patient had changes that were not specified.

Other serious AEs:

In the controlled clinical trials, 7% of the patients on 10 mg/day and 5% of patients on 5 mg/day or placebo had at least one serious AE. The serious AEs that were more frequent in the 10 mg/day group compared to placebo included: accidents, fractured bone, pain, hernia and aphasia. Accidents and fractured bones occurred at a frequency of 2% while the others occurred at a frequency of 1%.

In study 202, 34 of the 131 patients had at least one serious AE as of the cut off date for the NDA. The serious AEs occurring in more than one patient were cancers (7), agitation (5), stroke (2), seizure (2), fall (2), syncope (2) and low back pain (2). The AEs occurring in a single patient were hallucinations, duodenal ulcer, UTI, atrial arrhythmia, bronchitis, angina and renal colic.

In study 303, 67 of the 739 patients exposed to the drug, 196 of whom were

treated for > 50 weeks and 95% at doses of 10 mg/day, had at least one serious AE (including death) as the cut off date of 6/30/95. Serious AEs occurring in more than one patient were syncope (10), cancer (8), surgery (7), fall/fracture (6), UTI (5), pneumonia (4), atrial fibrillation (3), intracerebral bleed (3), MI (3), stroke (2), vomiting (2) and bronchitis (2) . Serious AEs occurring in a single patient were pulmonary embolism, viral encephalitis, hemoptysis, renal failure, abdominal pain, depression, sepsis, retinal vein thrombosis, agitation, cholecystitis, bleeding ulcer and agitation.

Treatment emergent AEs:

Approximately 95% of the all adverse events were rated as mild to moderate. Those occurring at a frequency of ≥ 5% in the 10 mg/day group and with a frequency greater than the placebo group are summarized in the following table.

Study 201, 301 and 302: Treatment Emergent Adverse events							
	Placebo (N=355)		5 mg (N=352)		10 mg (N=318)		5+10 mg (N=670)
	N	%	N	%	N	%	%
Nausea	19	5.35	20	5.68	59	18.55	11.79
Diarrhea	14	3.94	24	6.82	45	14.15	10.30
Insomnia	18	5.07	19	5.40	41	12.89	8.96
Headache	28	7.89	27	7.67	32	10.06	8.81
Pain	26	7.32	28	7.95	29	9.12	8.51
Dizziness	19	5.35	28	7.95	25	7.86	7.91
Accident	18	5.07	25	7.10	19	5.97	6.57
Muscle Cramp	6	1.69	19	5.40	24	7.55	6.42
Fatigue	11	3.10	13	3.69	24	7.55	5.52
Vomiting	9	2.54	10	2.84	25	7.86	5.22
Anorexia	5	1.41	9	2.56	18	5.66	4.03

Lab abnormalities:

For the controlled clinical trials, the mean lab values increased in the 5 and 10

mg group relative to placebo for the ALT, AST, LDH and CPK. The mean lab values decreased in the 5 and 10 mg group for glucose. There were no lab values where a shift in frequency was greater than 3% for the 10 mg group compared to placebo. For the long term extension study, 5% of the patients had an elevation of the CPK. The elevations in CPK did not appear to be related to any AE event or symptom.

Chemistry review:

The final chemistry reviews are pending at the time of this memo. From preliminary information, the sponsor needs to provide 3 month stability data from the US manufacturing site and an inspection needs to be completed at the Japanese site prior to chemists recommending that the drug be approved.

Pharm/tox review:

A review of the pharm/tox material was submitted by Barry Rosloff, Ph.D. He concluded that there were no issues that preclude approval of the drug. He recommended labeling changes.

Dr. Rosloff noted that although the carcinogenicity studies are ongoing, it has been policy for these studies to be submitted post marketing for drug used in the treatment of AD.

Biopharm review:

A biopharm review was submitted by VJ Tammara, Ph.D.

Dr. Tammara had requests for additional studies. The only study that was relevant to the approval of the drug was a dissolution study of the 5 and 10 mg tablets. All of the clinical studies were conducted using film coated 5 mg tablets manufactured in Japan. Patients on 10 mg/day took two 5 mg tablets The sponsor has proposed marketing both a 5 and a 10 mg tablet. The marketed dosage form will be made in the US. Studies demonstrated bioequivalency of the Japanese and US made 5 mg tablet. The sponsor is requesting a biowaver for the 10 mg tablet. According to Dr. Tammara, the sponsor must show similar dissolution profiles for the 5 and 10 mg tablet in order to obtain this biowaiver. These studies have not been performed. It is possible that the 10 mg tablet could lead to higher drug levels than two 5 mg tablets.

Dr. Tammara has recommended labeling changes. Most of the changes are related

to formatting. He has added additional information regarding metabolism and drug interactions. Dr. Tammara noted that the metabolism of e2020 may be affected by other drugs that are CYP 2D6 and CYP 3A4 inhibitors and inducers.

Additional issues:

The trademark ARICEPT is acceptable to the CDER Labeling and Nomenclature Committee (LNC) however, they noted that USAN objected to the use of the syllable CEPT in the ARICEPT name. USAN reserves this syllable for agents that act at specific receptors. The LNC points out that other CEPT trademarks have been approved for drugs that do not act at a specific receptor.

Conclusions:

Efficacy:

From recommendations of the PCNS advisory committee and experts in the field of AD, demonstration of a symptomatic effect in AD requires that a sponsor conduct placebo controlled, parallel, randomized, double blind trials of 3 and preferably 6 months in duration to demonstrate that the drug not only produce a significant improvement in cognitive function on a performance based test such as the ADAS-COG but that it also leads to a clinically detectable improvement. The results presented in the NDA demonstrates that the sponsor has accomplished these tasks. In two adequate and well controlled clinical trials, studies 301 and 302, the sponsor has shown that patients receiving e2020 have a statistically significant mean improvement in cognitive performance on the ADAS-COG and have a statistically significant mean clinical improvement as demonstrated on the CIBIC plus.

As seen with tacrine, another cholinesterase inhibitor, the size of the effect is modest. At 12 weeks, there appears to be a mean 2 to 3 point improvement in the ADAS-COG and 0.2 to 0.4 point improvement in the CIBIC plus. This treatment effect appears to persist with at least 24 weeks of treatment. The onset of improvement was first noted at 3 weeks, the first observation point, in study 201 and 301. In study 302, the groups were similar at 6 weeks, the first observation point, but different at the 12 weeks, the second observation point. The drug does not appear to have an effect on the underlying progression of the disease. This is suggested by the loss of cognitive function 3 to 6 week after discontinuing e2020 to a level seen in patients who were treated with placebo. The sponsor was unable to demonstrate a consistent effect in measures claiming to evaluate quality of life.

The sponsor also demonstrated that the treatment effect is dose related. In study 201, the cognitive performance of patients on doses of 1 and 3 mg/day were not significantly different from placebo whereas patients on 5 mg/day had a statistically significant improvement in ADAS-COG scores. While patients on 10 mg/day generally had numerically better scores than patients on 5 mg/day for the ADAS-COG and CIBIC, the differences were not statistically significant. In all cases, patients on both 5 and 10 mg/day had statistically significant improvement compared to placebo for both the ADAS-COG and CIBIC plus. The 5 mg/day group had better scored on the QOL measures compared to either the 10 mg/day group or the placebo group.

Safety:

The drug appears to be relatively well tolerated. Over 75% of patients in the highest dose group of 10 mg/day were able to complete the 3 to 6 month studies. Of the adverse events that occurred, 95% were rated as mild to moderate in severity. The most common problems with the drug were GI related, specifically, nausea, vomiting and diarrhea which is consistent with the expected cholinergic effects.

Some safety concerns for the cholinergic drugs such as GI ulcers, bradycardia, etc have been seen with this drug in small numbers of patients. While the incidence of these events do not suggest a causal relationship, precautions concerning the events seen for the drug class should be included in labeling.

Potential problems seen in the data base included falling and syncope. These were more common serious AEs though the cause is not known. An elevation of the CPK was also seen without clear cause or clinical significance.

In describing the safety of this drug, one drawback is the lack of long term experience, especially at the highest recommended dose of 10 mg/day. The NDA only contained complete safety information from 133 phase 2 patients treated long term with the drug. Since the maximum dose of the drug until late in phase 2 was 5 mg/day, there was essentially no experience at doses of 10 mg/day. The sponsor supplemented the safety data base with information on serious AEs and lab abnormalities in patients involved in the phase 3 long term extension studies, 303 and 305. In these studies, patients were allowed to be titrated to doses of 10 mg/day. In study 303, 95% of the patients were titrated to 10 mg/day. At the time of the cut off to the NDA, 196 patients were treated for about one year. Overall, in this long term experience, the pattern of serious AEs support the relative safety of this drug in patients with AD.

Recommendations:

Based on the findings of a statistically significant treatment effect along with relative safe passage, I recommend that NDA 20-690 is approvable pending final recommendations from the chemistry reviewers. Suggestions for changes to the sponsor's labeling from clinical, biopharm and pharm/tox are included in the attached document. Comments from biopharm review should be conveyed to the sponsor. Chemistry comments are pending. A safety update should be provided.

Randy Levin, M.D.
Medical Reviewer

cc:
Original IND
HFD-120
HFD-120/Leber/Higgins
rl/August 2, 1996

Final Printed Labeling

FINAL PRINTED LABELING HAS NOT BEEN SUBMITTED TO THE FDA.

DRAFT LABELING IS NO LONGER BEING SUPPLIED SO AS TO ENSURE ONLY CORRECT AND CURRENT INFORMATION IS DISSEMINATED TO THE PUBLIC.

Medical Officers Review

Review and Evaluation of Clinical Data

NDA:	**20-690**
Sponsor:	**Eisai**
Drug:	**e2020**
Indication:	
Material Submitted:	**Response to approvable letter**
Correspondence Date:	**10/3/96, 10/26/96**
Date Received:	**10/4/96**
Review Completed:	**11/9/96**

Introduction:

On 9/19/96, following review of the NDA submitted on 3/29/96, an approvable letter was sent to the sponsor. In the approvable letter, we provided draft labeling with comments and specific requests for the sponsor to address. We also requested a safety update. In the safety update, we requested that the sponsor provide a table that enumerates the dose and duration of patients exposed to the drug for each study and total. We also requested that the sponsor provide a table that compares the AEs at the time of submission and for the update. We requested that tables for drop outs identify new drop out and that the sponsor identify any significant changes or findings. We also requested electronic data sets for studies 202, 303 and 305, open label extension studies.

With the initial submission, the sponsor failed to provide information on the safety update as requested. They did not provide an adequate enumeration of the duration of exposure to each of the doses. They did not provide dosing information from the Japanese studies. The sponsor did not provided a comparison of the AEs and drop outs from the original study and the update. They did not provide electronic safety data sets for study 305. They did not provide the table of AEs stratified by dose group. This inadequate response significantly delayed the review of this response.

The problems with the safety update was conveyed to the sponsor and they submitted supplemental tables and files on 10/26/96

Safety update:

Introduction:

In the original NDA, the sponsor provided all safety information on the US controlled clinical trials, 201, 301 and 302 as well as study 202, the open label extension for study 201. They also provided information on the serious AEs, deaths and drop out from study 303, the open label extension for studies 301 and 302, study 304, an ongoing efficacy study and its open label extension, study 305. Finally information about serious AEs from Japanese phase 2 studies were included. The cut off dates for the labs was 12/95 and for AEs 9/30/95.

In the safety update, the sponsor has provided complete safety information for studies 202 and 303 as of the cut off date of 4/15/96. They have also provided information on labs, the serious AEs, deaths and discontinuations for studies 304 and 305.

Extent of exposure:

The sponsor failed to provide adequate documentation of the extent of exposure to 5 and 10 mg/day. They have not provided any information on the exposure to patients in study 304, 305 or the Japanese studies.

I have calculated the number of patients in studies 201, 202, 301, 302 and 303 exposed to 5 or 10 mg/day for 3, 6 or 12 months from the data sets provided by the sponsor in the NDA and update.

Number of patients exposed to e2020 for a specific duration in US studies (201, 202, 301, 302, 303)			
	5 mg/day	10 mg/day	Any dose
Study 303			
> 0 days	728	651	751
≥ 90 days	297	487	636
≥ 180 days	144	335	489
≥ 360 days	8	84	159
Study 202			
> 0 days	138	62	151
≥ 90 days	93	49	121
≥ 180 days	73	41	103
≥ 360 days	8	32	59
All US studies			
> 0 days	866	713	902
≥ 90 days	390	536	757
≥ 180 days	217	376	592
≥ 360 days	16	116	218

Number of patients exposed to e2020 for a specific duration in study 304*			
	5 mg/day	10 mg/day	Any dose
> 0 days	160	160	320
≥ 90 days	125	125	250
≥ 180 days	115	115	230

*Because study 304 is blinded and ongoing, the treatment assignments are not known. I have taken information from the chemistry data base on the number of patients with lab values at 90 and 180 days and divided the number by three based on the assumption that one third would be assigned to each treatment group.

The dose and duration for the phase 1 studies and the phase 2 Japanese studies (estimated) are provided in the following tables.

Dose and duration of exposure in phase 1 studies (some subjects may have received more than one dose)							
	Plb	0.1 to 1 mg	2 to 4 mg	5 mg	6 to 9 mg	10 mg	Total
One day	8	30	36	144	6	6	230
21 days	9	6	6	6			27
28 days				14		14	28
Total	17	36	42	164	6	20	285

Dose and Duration for the Japanese phase 2 studies						
	Plb	≤ 1 mg	2 mg	3 mg	5 mg	Total
8 weeks		31		9		40
12 weeks		33	31	2	18	84
16 weeks	60			66	64	190
38 weeks	19		19			38
48 weeks			46[a]			46
Total	79	64	96	77	82	398

[a]38 patients treated in study 131A were subsequently enrolled into study 131B.

Demographics:

The mean age of the patients was 73 with 15% < 65 and 5% ≥ 85. 42% were age 75 to 84 and 39% were age 65 to 74. 64% were female and 95% were white.

Discontinuations:

Study 202: 133 of the 141 eligible patients enrolled into study 202. As of 4/15/96, 38 remained in the study. 16 patients withdrew for AEs with two patients discontinuing since the NDA submission. Patient 042 withdrew on 120 mg/day withdrew for agitation after 1123 days on drug. Patient 092 received drug for 368 days and withdrew for increased confusion, agitation and hallucinations when

withdrawing on placebo.

Study 303: A total of 53 patients have discontinued from study 303. The reasons for discontinuation are summarized in the following table:

Reasons for discontinuation		
Reason	Number	Comments
GI	11	2 patients with bleeding ulcer
Agitation	10	
Syncope	9	Including near syncope, fainting
Stroke	3	
Dizziness	2	
MI	2	
Arrhythmia	3	Sinus bradycardia,
Other	12	One patient for each event

pancreatitis, lethargy, headache, incontinence, pneumonia, pulmonary embolism, cancer, meningitis, tremor, leg cramp, renal failure, death

Deaths:

As of 4/15/96, 34 patients have died while on treatment or within 4 weeks of discontinuing treatment. 8 have died since the NDA submission. These patients are described in the following table:

Patients who died since the NDA submission			
Patient		Reason	Comments
Study 202	071	MI and or PE	Treated 1159 days reached 10 mg/day
Study 303	212-301	Stroke	Treated 572 days reached 10 mg/day
	330-301	AD	Treated 491 days reached 10 mg/day
	450-301	AD	Treated 332 days on 5 mg/day
	475-301	Lung cancer	Treated 412 days reached 10 mg/day
	137-302	Lung cancer	Treated 419 days reached 10 mg/day
Study 305	578	Intracerebral hemorrhage	Treated for 5 days with 5 mg/day in open label following 6 month DB treatment

Serious AEs:

Study 202: 6 new cases of serious AEs occurred since the NDA submission.Two for pneumonia, one patient died with an MI or PE, one patient with lung cancer was hospitalized for dehydration, one patient with abdominal and lower back pain and one with hip fracture from a fall.

Study 303: Of the 761 patients exposed to the drug in study 303 (about 500 for 6 months or more, 152 serious AEs have been reported with 85 since the NDA submission. The most common serious AEs (number of patients) were cancer (17), GI problems (8), confusion/worsening AE (8), stroke (8), cardiovascular events (7) and syncope (7). A gross total of the serious AEs (no data set provided for the data since the NDA) included syncope (17), cancer (25), stroke including 3 intracerebral bleeds (13), cardiovascular events (10), GI problems including 2 UGI bleeds and one perforated ulcer (11), fall/fracture (12).

Study 304: 25 serious AEs were reported since the NDA. About one third of the serious AEs were related to increased agitation or confusion common event (8). One patient was diagnosed with cancer (malignant melanoma), one patient had a stroke and one patient had a syncopal episode.

Study 305: 17 serious AEs were reported since the NDA. One patient had an MI, one had a stroke, one had a TIA and one patient had an intracerebral

hemorrhage. Three patients had increase confusion.

Other AEs:

Study 202: 12 new AEs reported. 4 were for agitation.

Study 303: The most common AEs in study 303 are UTI (9%), pain (9%), agitation (9%), diarrhea (8%), accidents (8%). 19 patients had syncopal episode (2%). AEs seen in > 5% of patients in study 303 were seen with a similar frequency in patients in the controlled clinical trials.

When AEs were evaluated for the relationship to duration of treatment, the rate of UTIs, agitation and accidents increased during the later portions of the trials (weeks 25 to 60).

Vital signs:

Study 202: The only new clinically significant change in vital signs was recorded in a single patient, 037, who was noted to have a heart rate of 49.

Study 303: 6 patients had clinically significant changes in their vital signs. 2 patients had a decrease in pulse rate below 50, 1 had an increase in systolic BP, 1 had a decrease and two had an increase in diastolic BP.

ECGs:

Study 202: The sponsor reported in a separate communication that there were no changes in the ECG reports in study 202.

Study 303: Of 624 patients with at least one ECG during the study, 210 were noted to have sinus bradycardia. 64 had a heart rate of < 50. Only 5 patients had an ECG and pulse rate on exam < 50. 12 patients had clinically significant ECG abnormalities. 4 patients had sinus bradycardia, 2 had PVCs, 2 had prolongation of the QT interval (one was normal on repeat), 1 had atrial fibrillation, 1 had atrial flutter, 1 had t wave abnormalities, 1 had RBBB. 5 patients had ECGs that were serious or led to withdrawal. 2 had bradycardia associated with syncope, one had incomplete RBBB associated with syncope,1 had atrial fibrillation and one had an PVCs.

Labs:

Study 202: The sponsor reported that no clinically relevant changes in lab results were seen when compared to the NDA submission.

Study 303: The rates of abnormal labs were similar to rates reported in study 202 and the controlled studies in the ISS. There were no lab abnormalities reported as serious AEs. No lab abnormalities led to discontinuation of treatment except patient 346-301 who was found to have an elevated BUN and creatinine at week 6 while receiving 5 mg/day. The patient was subsequently discontinued for renal failure, hyperkalemia and bladder cancer.

There were 24 patients with an elevation in ALT, 6 of these patients also had an elevation of the Alk phos. All but 7 patients continued on treatment. Of the 7 patients who discontinued, none discontinued for the elevation. A single patient (511) had an elevation about 10 times the ULN. This patient had a single measurement and continued on drug for another 72 days prior to being discontinued for protocol violation. Two patients had elevations about 5 times the ULN and 2 had elevations about 3 times the ULN that either did not change over time with continued dosing or decreased with continued dosing.

An elevation in the CPK was seen in 149 patients. 36 of these patients had elevations at baseline. 17 patients with elevations discontinued treatment but the time of discontinuation was not associated with the elevation. 83 patients had elevations > 2 times baseline. Most of the elevations came down with continued treatment. There were no associated symptoms with the elevations.

Elevations of glucose was seen in abut 7% of patients similar to the elevations noted in the controlled clinical trials.

Hematology results did not appear to differ from the results seen in the controlled clinical trials. [redacted] of patients had a low hematocrit with 33% the lowest in males and 27.5% in females.

Study 304: As of 4/15/96, 604 patients had baseline lab evaluations and 317 patients had completed the 30 week study. Elevation in the CPK was seen in 10% of patients The elevation appears to have peaked at week 3. By week 24, the levels are similar to baseline.

Adverse events by age, sex and race:

There were slight increases in the rate of AEs seen with advancing age. Three AEs were seen with greater rates in females compared to males, accidents (5% v 9%), nausea (4% v 7%) and UTI (9% v 12%). Because there were only 36 non white patients, no analyses were presented by race.

Adverse events by titration:

In study 301 and 302, generally, there was an increase in AEs for patients randomized to 10 mg/day compared to those on 5 mg/day. In study 303, patients were titrated to 10 mg/day over 6 weeks instead of the 1 week titration in study 301 and 302. The incidence of AEs was generally less with this titration. I requested that the sponsor compare the AE rates for patients not previously exposed to drug with the one and six week titration schedule. Patients entering 303 who were assigned to placebo in studies 301 and 302 were compared to patients in study 301 and 302 assigned to drug. The rate of AEs was generally less in naive patients titrated to 10 mg over 6 week compared to those titrated over 1 week. This is summarized in the following table:

Comparison of rates of AEs in patients titrated to 10 mg/day over 1 week and over 6 weeks.					
	Study 301/302 (one week titration)			Study 303 (6 week titration)	
AE	Placebo (n=315)	5 mg/day (n=311)	10 mg/day (n=315)	301/302 Placebo patient (n=269)	AE rates for package insert (n=747)
Fatigue	3%	4%	8%	3%	5%
Diarrhea	5	8	15	9	10
Nausea	6	5	19	6	11
Vomiting	3	3	8	5	5
Anorexia	2	3	7	3	4
Wt loss	2	2	5	1	3
insomnia	6	6	14	6	9

Lab abnormalities by age, sex and race:

Elevated BUN and decrease hematocrit were more common in the older age groups. CPK levels were greater in the younger patients and males. Decrease hematocrit and calcium level abnormalities were seen more frequently in males.

Comments:

The sponsor has provided a safety update and revised labeling as requested in the approvable letter. The safety data base for which we have complete information is relatively small. In regards to the occurrence of serious AEs, the safety data base is larger in that it includes patients from study 304 and 305 and the data from safety update has not identified any new safety problems with the drug.

The sponsor has provided additional information regarding titration and adverse events. A comparison of patients treated with 10 mg/day following 1 week on 5 mg/day and 6 weeks on 5 mg/day indicated that the 10 mg dose was better tolerated following the 6 week titration.

In regards to labeling, after additional discussion with the sponsor, the following concerns were raised by the sponsor:

The sponsor wants to include a display of the CIBIC plus results over time. They argue that the addition of a graph depicting the CIBIC plus ratings over time is both useful to the prescriber and is scientifically justifiable. They describe the validity and reliability of the AD cooperative study consortium global and argue that the global has specific landmarks (anchor points). They have placed the figures showing the effects on the global over time. From the division's point of view, the CIBIC has been used only as a check on the ADAS-cog as an independent test on the efficacy of the drug. The reason that the ADAS-cog is discussed in such detail in the labeling is because it provides the prescriber with an indication of the modest effect of the drug. The CIBIC has less use for this purpose because it is not a standardized test like the ADAS-cog. The use of the CIBIC as proposed by the sponsor will not add any additional information not already provided by the ADAS-cog but can lead to confusion when prescribers review labeling for e2020 and tacrine as well as other drugs approved in the future. Inclusion of the CIBIC may lead to a false sense that e2020 is more effective than other drugs that do not include discussion of the CIBIC in labeling. For these reasons, we recommend not including the CIBIC time course in the labeling.

The sponsor wants to include more specific instructions on the use of the drug in the dosing section. Specifically, they want to identify the 5 mg dose as the initial dose. I have added this to labeling.

The sponsor has argued and I agree that the 1 week titration led to a different adverse event profile when compared to the 10 mg dose. I have included in labeling the most common AEs for both the 1 week and 6 week titration. Because the more rapid titration in the controlled trials appears to have added to the AEs reported for the 10 mg group, I have included in the AE table a comparison of the placebo and all doses of e2020 rather than the dosing by 5 and 10 mg group because this probably reflects more accurately the AE profile for patients taking the dose as recommended in the dosing section.

Recommendation:

The response and labeling has to be reviewed by all reviewers. From a clinical point of view, I recommend that the drug be approved with the attached labeling.

RdL

Randy Levin, M.D.
Neurology Team Leader

cc:
Original IND
HFD-120
HFD-120/Leber/Higgins
rl/November 14, 1996

REVIEW AND EVALUATION OF CLINICAL DATA

NDA:	**20-690 (000)**
SPONSOR:	**Eisai**
DRUG:	**Aricept (donepezil, e2020)**
INDICATION:	**Alzheimer's disease**
MATERIAL SUBMITTED:	**NDA**
CORRESPONDENCE DATE:	**3/29/96**
DATE RECEIVED:	**3/29/96**
DATE COMPLETED:	**7/1/96**

Introduction:

Aricept (donepezil, e2020) is a reversible acetylcholinesterase inhibitor (AChEI). It is a piperidine based inhibitor compared to tacrine which is a acridine based molecule.

Eisai submitted IND for the evaluation of e2020 in the treatment of Alzheimer's disease (AD) on 12/20/90. An end of phase 1 meeting took place on 9/17/91. An end of phase 2 meeting took place on 9/21/93. A pre NDA meeting took place on 11/13/95. The NDA was submitted on 3/29/96.

For this NDA review, I have used information from individual reports for studies 201, 301 and 302 as well as the integrated summary of safety and efficacy. This was provided as electronic documents from a CANDA submitted at the time of the NDA. I also used safety and efficacy data provided by the sponsor as part of the CANDA for additional review. I have also reviewed summaries of the preclinical data and pharmacokinetics (PK) data. I have also reviewed the sponsor's labeling proposal and provided comments and suggestions.

Other portions of the NDA were reviewed by Dr. Rzesotarski (chemistry), Dr. Rosloff (pharm/tox), Dr. Tammara (PK) and Dr. Hoberman (statistics).

Overview of the clinical data sources:

Phase 1:

Eighteen Phase 1 studies were conducted in Japan, UK and US and included PK studies, drug interaction studies and studies in special populations. A total of 285 subjects were enrolled with 262 receiving drug for up to 5 weeks. In phase 1 studies, the drug was found to be readily absorbed without significant first pass

metabolism. 95% is bound to human plasma proteins. The terminal half life was about 70 hours with steady state achieved in 15 days. The PK is linear from doses of 2 mg to 10 mg.

Phase 2:

Seven phase 2 studies were conducted in Japan. The study reports were not included in the NDA but serious AEs were reported in the integrated summary of safety. The safety results were reported by the sponsor to be similar to those seen in the US population. GI adverse events were most common. The studies are summarized in the following table:

Phase 2 studies conducted in Japan				
Study	Design	Doses studied (mg/day)	Duration of treatment	Number of patients
J081-111	Open label	1, 2	Up to 12 weeks	63
J081-112	Open label	3 mg	Up to 12 weeks	9
J081-131A	Open label	0.1, 2	Eight weeks	69
J081-131B	Open label	2	Up to 48 weeks	46
J081-132	Double blind	0, 2	24 to 48 weeks	38
J081-133	Open label	3, 4, 5	4 weeks per dose	20
J081-134	Double blind	3, 5	Up to 16 weeks	190

One phase 2 study, study 201, was conducted in the US. This was a 12 week, randomized, double blind, placebo controlled, parallel, study evaluating doses of 1, 3 and 5 mg/day. Patients completing this study were eligible to be enrolled into study 202, an open label extension. Following a series of protocol amendments, patients were allowed to be titrated to a maximum of 10 mg/day.

Phase 3:

Two phase 3 studies were conducted in the US, studies 301 and 302. Study 301 and 302 had similar designs. These studies were randomized, placebo controlled, double blind, parallel trials evaluating doses of 5 and 10 mg/day. In study 301, the double blind phase of the study was 12 weeks while in study 302, the duration of the double blind phase was 24 weeks. Patients were eligible for a long term extension study, 303. A study report for study 303 was not provided in the NDA. Deaths and serious AEs leading to withdrawal and lab values collected in study 303 prior to the cut off date are provided in the integrated summary of safety.

A third phase 3 study, 304, was ongoing as of the time of the NDA submission. It was being conducted outside the US and had a design similar to study 302 with doses of 5 and 10 mg/day being compared to placebo. 750 patients were to be enroll for this 24 week study. Patients were eligible for a long term extension, study 305. As of 12/31/95, 438 patients were enrolled in study 304. A study report was not provided with the NDA but deaths, serious AEs leading to withdrawal and lab values collected prior to the NDA cut off date were provided in the integrated summary of safety.

Overview of the pivotal and supportive efficacy studies

The sponsor has identified studies 301 and 302 as adequate and well controlled studies providing definitive evidence for the efficacy of e2020 in the treatment of AD. They have identified study 201 as a supportive trial.

A comparison of these three studies is summarized in the following table:

Comparison of protocols for studies 201,301 and 302			
Parameter	Study 201	Study 301	Study 302
Age of patients	55-85	≥50	≥50
Doses evaluated (mg/day)	0, 1, 3 and 5 mg/day	0, 5 and 10 mg/day	0, 5 and 10 mg/day
Patients per group	40/group	150 per group	150 per group
Duration of double blind phase	12 weeks	12 weeks	24 weeks
Duration of placebo washout	2 weeks	3 weeks	6 weeks
Primary efficacy outcome measures	ADAS-COG, CGIC	ADAS-COG, CIBI-C plus	ADAS-COG, CIBI-C plus
Secondary outcome measures	MMSE, QOL, ADL, CDR	MMSE, CDR, QOL	MMSE, CDR, QOL
Time points for efficacy assessments	0,1,3,6,9,12,14 weeks	0,1,3,6,9,12,15 weeks	0,6,12,18,24,30 weeks

Protocol:

Design:

In all three studies, patients were enrolled at multiple centers in the US. They were randomized in a double blind fashion to multiple doses of e2020 or placebo and treated in parallel for a duration of 12 to 24 weeks. The double blind treatment period was followed by a single blind, placebo washout and patients were then eligible for open label extension studies.

Dosing:

In study 201, the patients received tablets containing 0, 1, 3 or 5 mg of the drug. The tablets were given once each evening prior to bedtime. In study 301 and 302, 5 mg tablets were used and patients received either 0, 5 or 10 mg each evening prior to bedtime. Patients on 10 mg received 5 mg/day for 7 days and then 10 mg for the duration of the study. The titration was blinded.

Selection:

Ambulatory patients with probable Alzheimer's disease (AD) as defined by the NINCDS and DSM-III-R criteria who were generally in good health were enrolled. Patients had mild to moderate AD as defined by an MMSE score of 10 to 26, inclusive, and a CDR rating of 1 or 2. Patients were excluded if they had insulin dependent diabetes or uncontrolled non insulin dependent diabetes, chronic obstructive pulmonary disease or asthma, clinically significant, active gastrointestinal, renal, hepatic, endocrine or cardiovascular system disease. Patients with controlled hypertension (supine diastolic BP < 95 mmHg), right bundle branch block (complete or partial) and pacemakers were allowed. Patients with schizophrenia, delirium, delusions and depression were excluded. Patients with dementia complicated by other organic disease were excluded.

Outcome variables:

ADAS-COG: The ADAS-COG used in these studies were similar to those used in the evaluation of Cognex. The 11 item portion of the 21 item ADAS evaluating memory, orientation, language and praxis was used. Scores could range from 0 to 70. Worsening is associated with an increase in score .

CIBIC plus: This was used in study 301 and 302. This was a global rating similar to that used in the evaluation of Cognex. In this rating, a clinician familiar with AD, interviewed the patients and the patient's caregiver in a semi structured interview. The raters were directed to inquire about areas of functioning in the domains of general, cognitive and behavioral functioning as well as activities of daily living This clinician was not involved with the patient's care and was blinded to performance tests scores and adverse events (AEs) experienced by the patient. The clinician evaluated the patients at baseline and then rated the impression of change based on a 7 point scale. A rating of 4 equaled no change. A rating of 1, 2 and 3 equaled marked, moderate and minimal improvement respectively. A rating of 5, 6 and 7 equals minimal, moderate and marked worsening, respectively.

CGIC: This scale was only used in study 201. The clinician, who was blinded to the ADAS-COG and MMSE score only, based their assessment on their clinical experience with the patient. On a 7 point scale, without anchor points, they would rate the global impression of change and the severity of the illness.

MMSE: This test, commonly used as a screening tool for cognitive function, was based on 11 questions assessing orientation, memory and attention, the ability to name objects, follow verbal and written command and copy. The maximum score was 30 with a higher score associated with better cortical functioning.

Clinical Dementia Rating (CDR): This was a test to rate the stages of dementia by assessing 6 functional categories: memory; orientation; judgement and problem solving; community affairs; home and hobbies, and personal care. The rating was performed by members of the patient's treatment team, except for the CIBI-C interviewer. Raters interviewed the patients and had access to collaborating sources including results of all of the other tests. The ratings were on a scale from 0 to 3 based on the severity of the AD. No AD (CDR 0), questionable or uncertain AD (CDR 0.5), mild AD (CDR 1), moderate AD (CDR 2) or severe AD (CDR 3). During the study, scores from each of the 6 categories are added (Sum of Boxes) to provide a total score.

Uniform Activities of Daily Living (ADL): This scale had 10 sections evaluating the patient's abilities in: bathing, grooming, dressing/undressing, feeding, toileting, movement, telephone, errands, table setting and miscellaneous activities. A total of 76 items were represented on the scale and patients were evaluated on an 8 point scale: 1 = independent, 2 = requires assistance, 3=requires supervision, 4 = requires assistance, but no lifting, 5 = requires assistance and physical lifting, and 8 = can not do. High scores on the ADL represented a greater degree of impairment.

Quality of Life (QOL): In study 201, there were 8 items and in studies 301 and 302, there were 7 items to evaluate the patient's perception of well-being, as measured by relationships, eating and sleeping and social/leisure activity. This test was conducted through an interview with the patient by a nurse/evaluator or clinician. For study 201, a separate interview was conducted with the caregiver to determine their perception of the patient's life quality.

Analysis:

Definitions: (1) "On treatment" visit were all visits that occurred prior to or no

more than 3 days after the last double blind dose. Otherwise the treatments were considered "off treatment". (2) Endpoint visit was defined as the last available post baseline assessment obtained from an on treatment visit. If an on treatment assessment was not available then the last available off treatment assessment was used. In study 201 and 301, the endpoint visit was week 12, in study 302, the Endpoint visit was Week 24. (3) Retrieved drop out visit was defined an off treatment visit at week 12 for study 301 and week 24 for study 302.

Missing efficacy scores: Missing variables were not replaced in these studies. Total scores were not calculated if one or more subcomponents were missing. If a patient was unable or unwilling or cooperate with a test, then the patient was assigned the worst score.

Patient populations:

(1) Observed cases were all patients randomized to treatment, received at least one dose of medication, had baseline data, and had an on treatment assessment at week 12 in study 201 and 301 or week 24 in study 302, irrespective of compliance and protocol violation.

(2) LOCF included all patients randomized to treatment, who received at least one dose of medication, had baseline data, and had at least one post baseline, on treatment assessment, irrespective of compliance and protocol violation. The last available on treatment assessment was used.

(3) Classical intent to treat included all patients randomized to treatment, who received at least one dose of medication, had baseline data, and had at least one post baseline assessment, irrespective of compliance and protocol violation. For patients who did not have a week 12 or week 24 assessment, the retrieved drop out score was used. For patients without the week 12 or week 24 on or off treatment assessment, the last observation was used.

(4) The Fully Evaluable subset was defined as all patients who went through 12 or 24 weeks of double blind treatment, were $\geq$ 80% compliant and had at least two other visits during the double blind treatment and no significant protocol violations such as disallowed concomitant medications. Assessments had to be made within 3 days of taking the study treatment.

Primary analysis: The primary analysis was the endpoint data for the Fully Evaluable population.

Model: For continuous variables, the comparison was based on the change from baseline to the Endpoint visit using ANCOVA including effects for treatment, site and treatment by site interaction with the baseline as a covariate. For the CIBIC plus, the treatment group comparison was analyzed using CMH test with the centers as strata. Scores of 0 were not used in the analysis.

Results:

Studies 301 and 302 provided evidence for improvement in cognitive functioning as measured by the ADAS-COG with demonstration of global improvement as measured by the CIBIC. Study 201 provided supportive evidence. The results are summarized in the following table:

Summary of efficacy results (treatment effect for the highest e2020 dose[1] vs placebo using LOCF data set[2])			
Outcome measure	Study 201	Study 301	Study 302
ADAS-COG	* (-3.19)	* (-3.07)	* (-2.88)
CIBIC plus	n/a	* (-0.34)	* (-0.44)
CGIC	+ (-0.11)	n/a	n/a
MMSE	+ (1.11)	* (1.82)	* (1.36)
CDR	+ (-0.21)	+ (-0.17)	* (-0.6)
ADL	+ (-4.61)	n/a	n/a
QOL	- (3.34)	n/a	- (-2.05)

[1]Highest dose for study 201 was 5 mg while 10 mg was the highest dose for study 301 and 302.
[2]LS means used for treatment differences in all cases except for the CIBIC and CGIC
*Statistically significant in favor of e2020 when compared with placebo
+ No statistical difference but numerical difference in favor of the drug
- No statistical difference but numerical difference in favor of placebo

Figure A

Mean Change from Baseline for ADAS-cog by Assessment and Treatment Group: Study 301

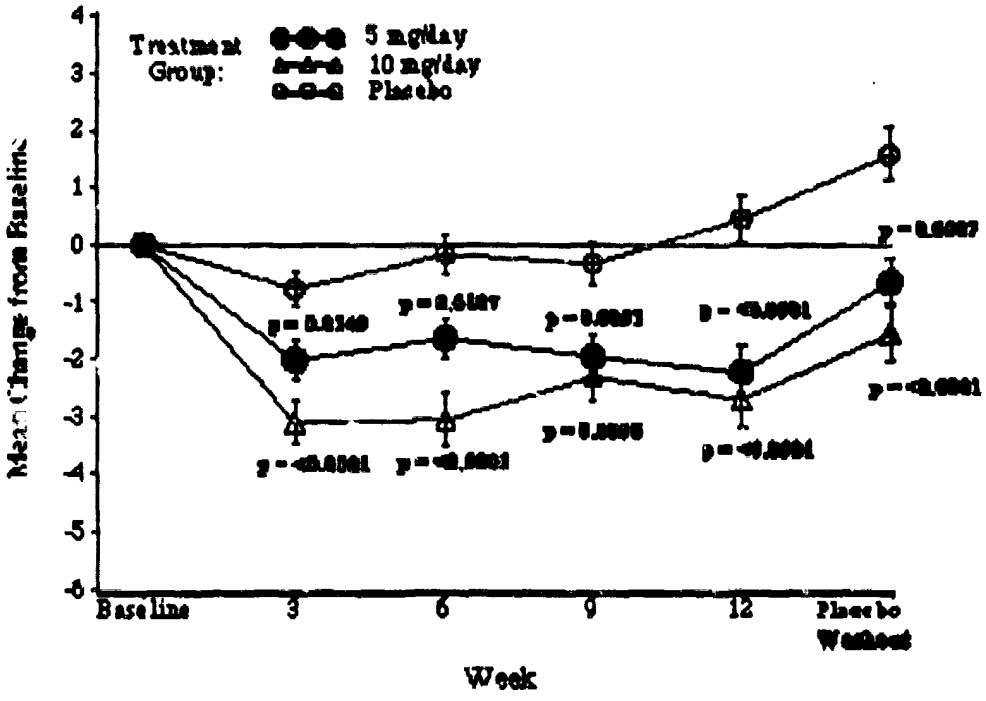


Figure C

Mean Change from Baseline for ADAS-cog
By Assessment and Treatment Group: Study 302

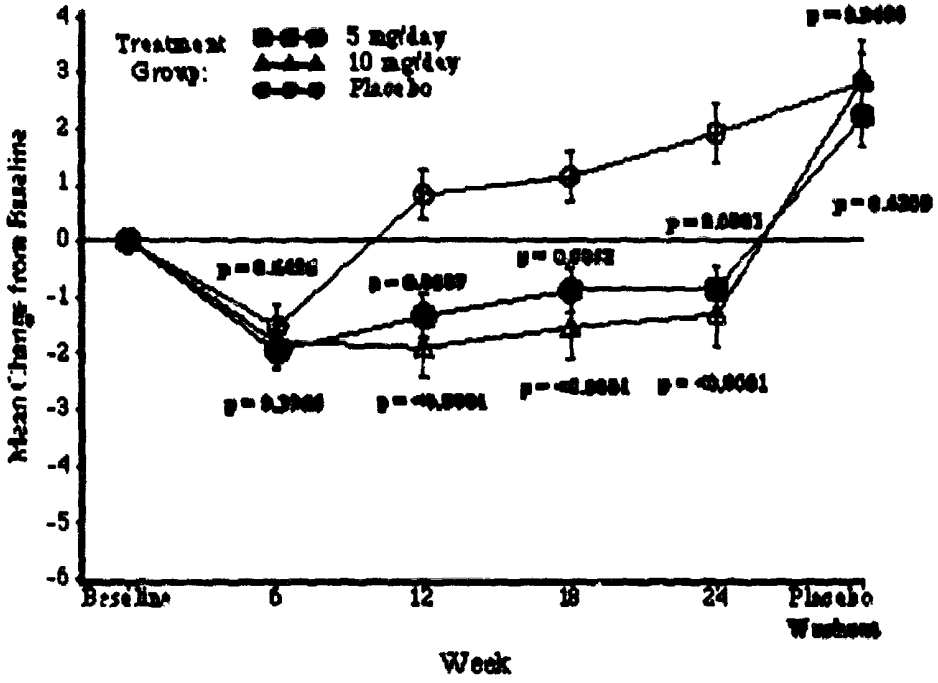
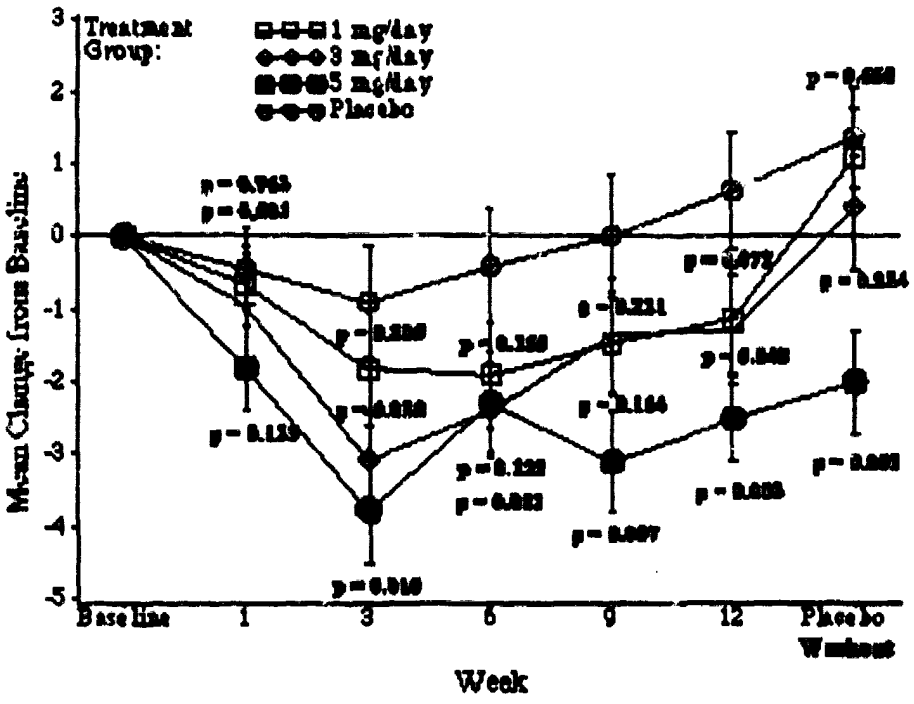


Figure E

Mean Change from Baseline for ADAS-cog by Assessment and Treatment Group: Study 201



Efficacy results for study 302:

Disposition:

32% of the patients on 10 mg/day dropped out compared to 20% of patients on placebo. AEs were the major reason for dropping out. The disposition of patients is summarized in the following table:

Study 302: Disposition of patients			
	Placebo	5 mg	10 mg
Number of patients at each visit (%)			
Baseline	162	154	157
week 6 (%)	146 (90)	144 (94)	137 (87)
week 12 (%)	139 (86)	139 (90)	126 (80)
week 18 (%)	138 (85)	135 (88)	113 (72)
week 24 (%)	130 (80)	131 (85)	106 (68)
Discontinued (%)	32 (20)	23 (15)	51 (32)
AE (%)	11 (7)	9 (6)	26 (17)
Request (%)	5 (3)	4 (3)	5 (3)
non compliance (%)	0	2 (1)	7 (4)
protocol violation (%)	7 (4)	2 (1)	7 (4)
other (%)	9 (6)	6 (4)	8 (5)

Demographics:

The demographics and baseline characteristics of the treatment groups were similar. The only difference that reached statistical significance was age. In the statistical analysis treatment by age interaction was not significant. Inclusion of age in the model, did not alter the statistical significance of the findings. The mean age was 73, 62% of the patients were female. 95% were white. 75% had a CDR rating of 1 and the mean MMSE score was 19.

Primary outcome measures:

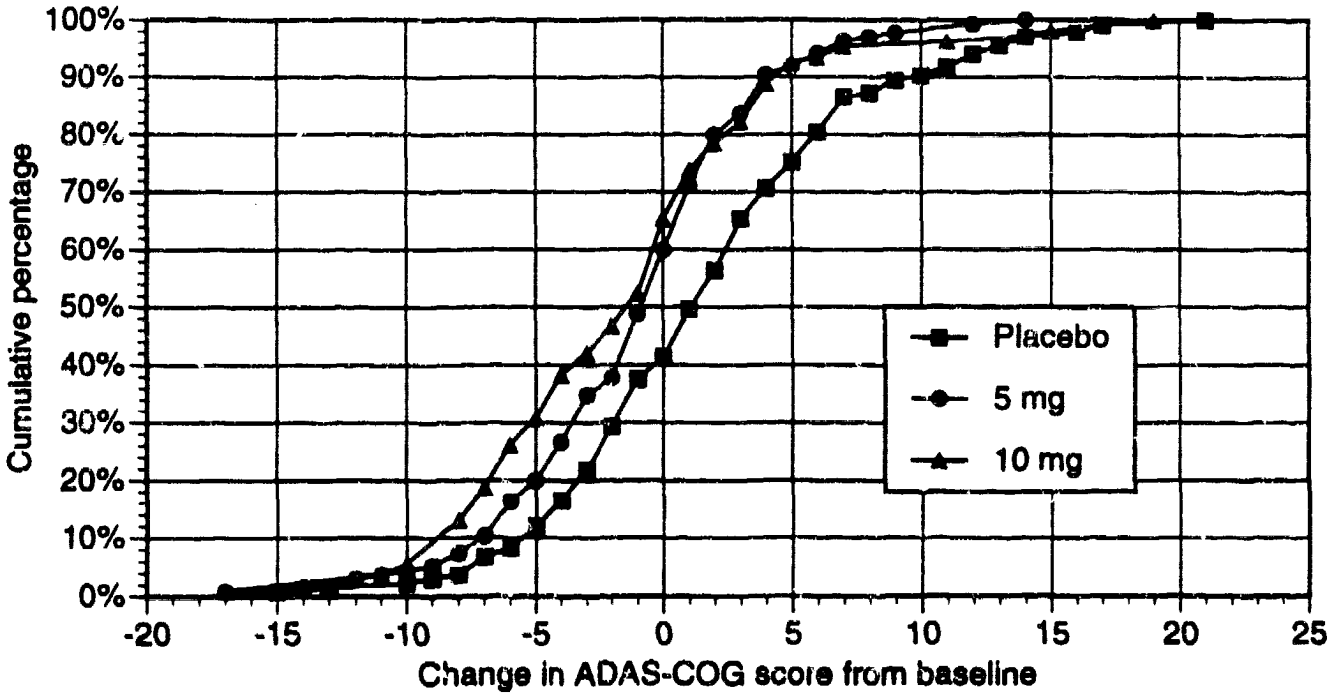
ADAS-COG: The treatment differences were statistically significant at week 12 through week 24. The results of the ADAS-COG are summarized in the following table:

Study 302: ADAS-COG change from baseline (± SE)			
	Placebo	5 mg/day	10 mg/day
Baseline	27.3 (0.34)	26.1 (0.35)	27.4 (0.31)
Observed cases			
week 6	-1.51 (0.39)	-1.86 (0.35)	-1.83 (0.45)
week 12	0.80 (0.42)	-1.25 (0.41)	-1.97 (0.50)
week 18	1.90 (0.51)	-0.90 (0.41)	-1.48 (0.56)
week 24	1.91 (0.52)	-0.78 (0.42)	-1.26 (0.57)
week 30	2.82 (0.53)	2.70 (0.57)	1.89 (0.53)
LOCF	1.91 (0.49)	-0.69 (0.40)	-1.40 (0.51)
ITT	1.90 (0.49)	-0.61 (0.41)	-1.07 (0.50)

There was a significant treatment by site interaction that appeared to be related to magnitude and not direction. In 22 of 24 sites, either the 5 or 10 mg/day group had better scores compared to placebo. In 15 of 24 sites, both the 5 and 10 mg/day group had better scores compared to placebo.

I took the week 24 observed cases and rounded the change in ADAS-COG score from baseline to the nearest whole number and plotted the cumulative frequency results in the following figure:

Cumulative percentage of patients with changes from baseline ADAS-COG score (24 week observed cases data set)



CIBIC plus: There were statistically significant differences between the active groups and placebo at 12, 18 and 24 weeks. The results of the CIBIC plus are summarized in the following table:

Study 302: CIBIC plus score (± SE)			
	Placebo	5 mg/day	10 mg/day
Observed cases			
week 6	3.97 (0.066)	3.94 (0.065)	3.96 (0.069)
week 12	4.20 (0.075)	3.99 (0.066)	3.92 (0.085)
week 18	4.40 (0.083)	4.10 (0.082)	3.94 (0.093)
week 24	4.53 (0.084)	4.20 (0.093)	4.09 (0.099)
week 30	4.75 (0.087)	4.45 (0.097)	4.65 (0.086)
LOCF	4.50 (0.080)	4.18 (0.091)	4.07 (0.086)
ITT	4.52 (0.080)	4.18 (0.091)	4.07 (0.086)
ITT Total N	162	157	153
ITT Score1	0	2	1
2	6	5	2
3	9	30	29
4	55	48	60
5	55	44	33
6	19	12	7
7	1	1	1

By week 12, the difference between the 10 mg/day dose and placebo was associated with a p value of < 0.05. On week 18 and 24, both the 5 and 10 mg/day doses were associated with a p value of < 0.05 when compared to placebo. Similar results were seen with the LOCF and ITT data sets. 14 of 24 sites had a better score in both the treatment groups compared to placebo. In 22 of 24 sites, either the 5 or 10 mg/day dose had a better score than placebo.

Responders:

I defined a responder as a patient with no change or better on the week 24 CIBIC plus and no change or better on the week 24 ADAS-COG. 61/161 (38%) patients on placebo, 71/153 patients (46%) on 5 mg/day and 100/157 patients (64%) on 10 mg/day fulfilled these criteria. The differences between the 10 m/day dose and

placebo was associated with a nominal p value of < 0.05.

Secondary outcome measures:

The sponsor collected information on the MMSE, CDR and QOL. An increase in score represents improvement for the MMSE and QOL and a deterioration for the CDR. Patients on both 5 and 10 mg/day had improvement in MMSE and CDR scores that were associated with nominal p values < 0.001. The difference between groups for the QOL were associated with p values > 0.05. The direction of the scores were in favor of the drug for the 5 mg/day group when compared to placebo and in favor of the placebo group when compared to the 10 mg/day group.

The treatment by site interaction was significant for the endpoint CDR and MMSE. The sponsor stated that it appeared to be based on magnitude of improvement rather than direction. The treatment by baseline interaction was also significant for the CDR. This appeared to be a result of greater improvement seen in the 10 mg/day group in patients with higher baseline scores.

The results of the LS mean change scores for the week 24 ITT data set are summarized in the following table:

Secondary outcome measures: LS mean change from baseline				
		Placebo	5 mg/day	10 mg/day
MMSE	Baseline	19.40 (0.37)	19.44 (0.37)	19.17 (0.37)
	Week 24 ITT	-0.97 (0.28)	0.24 (0.28)***	0.39 (0.29)***
CDR	Baseline	6.98 (0.19)	7.11 (0.19)	7.13 (0.19)
	Week 24 ITT	0.58 (0.14)	-0.03 (0.14)***	-0.02 (0.14)***
QOL	Baseline	295 (3.35)	287 (4.03)	282 (3.91)
	Week 24 ITT	-2.8 (3.18)	3.4 (3.27)	-4.9 (3.29)

***P value < 0.001, **p value < 0.01, *p value < 0.05 in comparison with placebo.

Efficacy results for study 301:

Disposition:

18% of the patients on 10 mg/day dropped out compared to 7% of patients on placebo. AEs were the major reason for dropping out. The sponsor excluded 13 patients from investigator 53 in Hartford because of errors in record keeping. The disposition of patients is summarized in the following table:

Study 301: Disposition of patients			
	Placebo	5 mg	10 mg
Number of patients at each visit			
Baseline	153	157	158
week 3 (%)	150 (98)	149 (95)	146 (92)
week 6 (%)	147 (96)	145 (92)	137 (87)
week 9 (%)	145 (95)	142 (90)	131 (83)
week 12 (%)	142 (93)	142 (90)	131 (83)
Discontinued (%)	11 (7)	16 (10)	29 (18)
AE (%)	3 (2)	7 (4)	16 (10)
Request (%)	3 (2)	4 (3)	6 (4)
non compliance (%)	1 (1)	0	0
protocol violation (%)	2 (1)	3 (2)	4 (3
other (%)	2 (1)	2 (1)	3 (2)

Demographics:

The demographics and baseline characteristics of the treatment groups were similar. The only difference that reached a nominal p value < 0.05 was race though $\geq 95\%$ of the patients were white. The mean age was 74, 63% of the patients were female. >75% had a CDR rating of 1 and the mean MMSE score was 19.

Primary outcome measures:

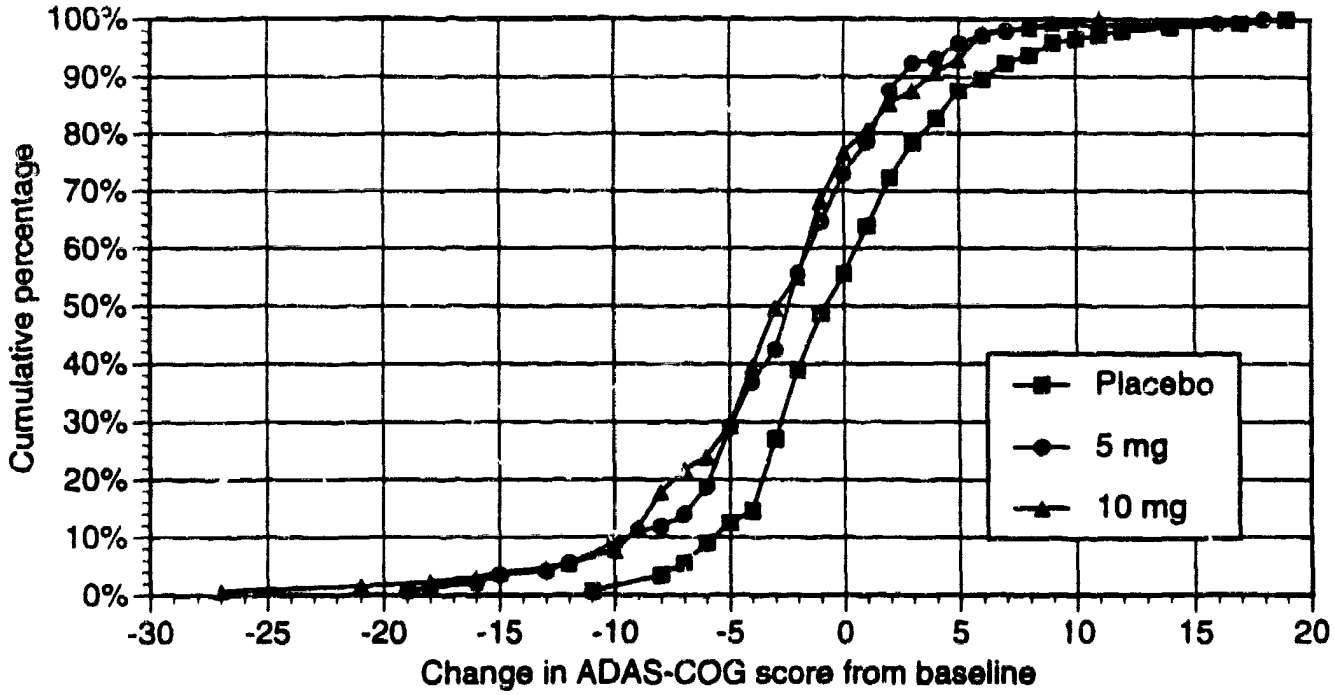
ADAS-COG: The patients in the 5 and 10 mg/day groups demonstrated a statistically significant difference in the change score of the ADAS-COG at all time points during treatment. The scores of the 10 mg/day group were numerically better than the 5 mg/day group though there was no statistical difference between groups. Following withdrawal, there continued to be a statistically significant difference between drug and placebo groups. The results are summarized in the following table:

Study 301: ADAS-COG change from baseline (±SE)			
	Placebo	5 mg/day	10 mg/day
Baseline (±SE)	25.7 (0.82)	26.2 (0.88)	26 6 (0.87)
Observed cases			
week 3	-0.69 (0.30)	-1.98 (0.36)	-3.03 (0.36)
week 6	-0.14 (0.35)	-1.55 (0.34)	-3.01 (0.45)
week 9	-0.27 (0.36)	-1.98 (0.39)	-2.52 (0.44)
week 12	0.45 (0.41)	-2.22 (0.45)	-2.68 (0.50)
week 15	1.5 (0.45)	-0.57 (0.41)	-1.61 (0.47)
LOCF	0.44 (0.39)	-2.12 (0.43)	-2.69 (0.47)
ITT	0.42 (0.39)	-2.17 (0.43)	-2.64 (0.47)

There was a significant treatment by site interaction that the sponsor noted to be related to magnitude and not direction. In all sites, either the 5 or 10 mg/day group had better scores compared to placebo. In 20 of 24 sites, both the 5 and 10 mg/day ADAS-COG scores were better than placebo. There was no difference in significance when the data was analyzed by the sex of the patient. There was a statistically significant treatment by baseline interaction. The sponsor noted that this was a results of a greater improvement in patients with higher baseline values in the 10 mg/day group.

Cumulative percentage of patients with a change from baseline in the ADAS-COG is summarized below:

Cumulative percentage of Patients with a specific change from baseline fjor the ADAS-COG (12 week observed cases)



CIBIC plus: The scores of the patients receiving 5 and 10 mg/day were statistically better than patients on placebo by week 12 (p values ≤ 0.01). At week 9, there was a statistically significant difference between the patients on 5 mg/day and placebo. At 15 weeks, the groups were not significantly different. Similar results were seen with the LOCF and ITT data sets. The results are summarized in the following table:

Study 302: CIBIC plus scores (± SE)			
	Placebo	5 mg/day	10 mg/day
Observed cases			
week 3	3.98 (0.052)	3.98 (0.053)	3.86 (0.062)
week 6	3.98 (0.066)	3.84 (0.068)	3.91 (0.068)
week 9	4.03 (0.076)	3.74 (0.082)	3.84 (0.068)
week 12	4.19 (0.074)	3.85 (0.081)	3.82 (0.082)
week 15	4.25 (0.080)	4.07 (0.088)	4.10 (0.088)
LOCF	4.20 (0.072)	3.87 (0.078)	3.82 (0.079)
ITT	4.21 (0.072)	3.87 (0.079)	3.82 (0.079)
Total N	154	151	143
Score 1	0	0	1
2	5	12	8
3	22	38	29
4	73	66	54
5	45	29	32
6	8	5	3
7	1	1	0

In 21 of 24 sites, the CIBIC plus score was better in either the 5 or 10 mg/day group or both compared to placebo. The sex by treatment interaction was not statistically significant. The mean proportions of visits with a score of > 4 ("failed visit") was statistically different between patients treated with 5 mg/day and placebo but not for patients on 10 mg/day.

Secondary outcome measures:

The sponsor collected information on the MMSE, CDR and QOL. An increase in score represents improvement for the MMSE and QOL and a deterioration for the CDR. Patients on both 5 and 10 mg/day had improvement in the MMSE that was associated with p values < 0.01. There was no difference between groups for

the CDR. The difference between groups for the QOL were associated with p values > 0.05. For the QOL measure, the direction of the scores were in favor of the drug for the 5 mg/day group when compared to placebo and in favor of the placebo group when compared to the 10 mg/day group (p =0.025).

The treatment by site interaction was significant for the endpoint CDR and MMSE. The sponsor noted that this appeared to be based on magnitude of improvement rather than direction.

The results of the LS mean change scores for the week 24 ITT data set are summarized in the following table:

Study 301: Secondary outcome measures: LS mean change from baseline (± SE)				
		Placebo	5 mg/day	10 mg/day
MMSE	Baseline	19.80 (0.35)	19.39 (0.39)	19.35 (0.40)
	Week 12 ITT	0.14 (0.26)	1.03 (0.25)**	1.35 (0.2)***
CDR	Baseline	6.81 (0.18)	6.85 (0.18)	7.18 (0.20)
	Week 12 ITT	-0.14 (0.11)	-0.10 (0.11)	-0.31 (0.11)
QOL	Baseline	289 (3.44)	292 (3.58)	284 (3.51)
	Week 12 ITT	4.0 (2.73)*	5.7 (2.70)	-4.27 (2.71)

***P value < 0.001, **p value < 0.01, *p value < 0.05 in comparison with placebo.

Responders:

I defined a responder as a patient with no change or better on the week 24 CIBIC plus and no change or better on the week 24 ADAS-COG. 57 (40%) patients on placebo, 91 patients (64%) on 5 mg/day and 79 patients (61%) on 10 mg/day fulfilled these criteria.

Efficacy results study 201:

Disposition:

The disposition of patients is summarized in the following table:

Study 201: Disposition of patients				
	Placebo	1 mg	3 mg	5 mg
Number of patients at each visit				
Baseline	40	42	40	39
week 3 (%)	37 (93)	39 (93)	40 (100)	38 (97)
week 6 (%)	36 (90)	37 (88)	40 (100)	35 (90)
week 9 (%)	35 (88)	36 (86)	39 (98)	35 (90)
week 12 (%)	36 (90)	35 (83)	38 (95)	36 (92)
Discontinued (%)	5 (13)	8 (19)	2 (5)	5 (13)
AE (%)	2 (5)	5 (12)	2 (5)	3 (8)
Request (%)	0	1 (2)	0	0
non compliance (%)	0	0	0	0
protocol violation (%)	3 (8)	1 (2)	0	1 (3)
other (%)	0	1 (2)	0	1 (3)

Demographics:

The demographics and baseline characteristics of the treatment groups were similar. The only differences that was associated with a nominal p value < 0.05 were height and weight though the actual differences were small. The mean age was 70 to 73 years. 28 to 45% of the patients in the groups were male. ≥ 90% of the patients were white. The mean MMSE score at baseline was 17.8 to 19.3.

Primary outcome measures:

ADAS-COG: The patients in the 5 mg/day group demonstrated a statistically significant difference in the change score of the ADAS-COG at week 3, 9 and 12. There was no statistical difference

between the 1 and 3 mg/day groups and placebo. Following withdrawal, there continued to be a statistically significant difference between the 5 mg/day group and placebo groups. The LOCF analysis had the same results. In this study drop outs were not retrieved so an ITT analysis was not performed. The results are summarized in the following table:

Study 201: ADAS-COG change from baseline (±SE)				
	Placebo	1 mg/day	3 mg/day	5 mg/day
Baseline (±SE)	27.2 (1.78)	26.6 (1.52)	29.2 (1.71)	29.1 (2.00)
Observed cases				
week 3	-0.91 (0.77)	-1.82 (0.80)	-3.09 (0.66)	-3.77 (0.75)
week 6	-0.42 (0.79)	-1.92 (0.73)	-2.40 (0.66)	-2.30 (0.70)
week 9	0.01 (0.83)	-1.46 (0.71)	-1.35 (0.78)	-3.11 (0.69)
week 12	0.64 (0.82)	-1.12 (0.76)	-1.28 (0.76)	-2.52 (0.59)
week 14	1.37 (0.70)	1.10 (0.68)	0.41 (0.87)	-2.00 (0.71)
LOCF	0.66 (0.75)	-0.87 (0.68)	-1.33 (0.72)	-2.43 (0.54)

Additional analyses were performed to correlate the ADAS-COG scores with plasma levels (r=-0.198, p value 0.014), inhibition of AChEI (r=-0.216, p=0.008), CGIC (p < 0.001), ADL (p=0.052), QOL (p=0.261), CDR (p=0.002) and MMSE (p< 0.003).

CGIC: This study was designed prior to recommendations about conducting global evaluations. There were no statistically significant differences noted between groups for the CGIC for the observed case or LOCF data sets. The results are summarized in the following table:

Study 201: CGIC scores (±SE)				
	Placebo	1 mg/day	3 mg/day	5 mg/day
Observed cases				
week 0	3.98 (0.025)	3.81 (0.13)	4.05 (0.05)	4.03 (0.045)
week 3	3.76 (0.098)	3.92 (0.093)	3.85 (0.098)	3.80 (0.080)
week 6	3.92 (0.14)	3.78 (0.088)	3.75 (0.10)	3.80 (0.11)
week 9	3.91 (0.14)	4.06 (0.13)	3.77 (0.10)	3.74 (0.10)
week 12	3.81 (0.15)	3.83 (0.12)	3.86 (0.11)	3.72 (0.12)
week 14	4.11 (0.16)	4.05 (0.11)	3.92 (0.12)	3.89 (0.11)
LOCF	3.85 (0.15)	3.88 (0.11)	3.90 (0.11)	3.74 (0.11)
Total N	40	41	39	38
Score 1	0	0	0	0
2	3	1	0	0
3	12	10	8	12
4	18	23	24	21
5	6	7	6	4
6	2	0	0	0
7	0	0	0	0

A comparison of the mean proportions of visits with a score of > 4 ("failed visit") between patients treated with 5 mg/day and placebo was associated with a nominal p value of 0.072.

Secondary outcome measures:

The sponsor collected information on the ADL, MMSE, CDR and QOL. An increase in score represents improvement for the MMSE and QOL and a deterioration for the ADL and CDR. For the ADL, the 5 mg/day group had better scores compared to placebo but the changes were associated with a nominal p value of 0.168. In the MMSE, all centers reported improvement for the 5 mg/day group but the changes were associated with a p value of 0.155. For the QOL measure, the differences between the 5 mg/day group and placebo was

associated with a p value of 0.21. Analysis of dose response was associated with a p value of 0.0383 for the patient rated QOL. In all of the comparisons involving secondary outcome measures, there were no treatment differences associated with a p value of < 0.05.

Study 201: Secondary outcome measures: LS Mean change from baseline (week 12 LOCF data set used)					
		Placebo	1 mg/day	3 mg/day	5 mg/day
ADL	Baseline	92.44	91.9	98.8	105.6
	LOCF	1.5 (2.38)	4.0 (3.10)	0.58 (1.56)	-3.07 (1.67)
MMSE	Baseline	18.2	19.8	18.6	17.8
	LOCF	1.15 (0.38)	0.56 (0.40)	0.89 (0.42)	1.99 (0.32)
CDR	Baseline	6.66	6.48	6.91	7.30
	LOCF	0.1 (0.17)	0.18 (0.19)	0.23 (0.21)	-0.11 (0.15)
QOL	Baseline	295	291	270	283
	LOCF	-1.33 (5.20)	0.75 (4.83)	2.56 (7.00)	8.75 (7.18)

***P value < 0.001, **p value < 0.01, *p value < 0.05 in comparison with placebo.

Sponsor's conclusions:

The sponsor offered the following as a conclusion for the efficacy results:

> All three controlled studies demonstrate that E2020 significantly improves cognitive functioning. These findings are supported by statistically significant improvement as measured by the ADAS-COG. This finding was consistent across the three studies and is supported by data generated not only for the ADAS-COG but also for the MMSE (in Study 301 and 302).
>
> The results of Study 201 generally support those found in Studies 301 and 302 on tests of cognitive status. The ADAS-COG measure was statistically significantly improved by E2020 relative to placebo and there was a trend toward improvement on the MMSE.
>
> The conclusion of all three studies with respect to the effect of E2020 on the cognitive status in Alzheimer's Disease is the same: E2020 improves functioning on measures of cognitive ability in dementia patients. This effect is strong statistically and clinically and is consistent across studies.
>
> Evidence for general clinical global improvement during the treatment period was collected

by conducting evaluations of patients' global functionality. These evaluations were performed by clinicians who were blinded to the outcome of cognitive testing and other test procedures such as laboratory results and adverse event reports. Interviews with the patient and a collateral source who had knowledge of the patient's general functioning were conducted.

In Studies 301 and 302, consistent and statistically significant improvements on the CIBI-C Plus were reported. Patients receiving E2020 were less likely than placebo treated patients to exhibit a worsening in clinical condition at any assessment and were more likely to show clinical improvement. The results of the CDR-SB support those of the CIBI-C Plus.

The small dose-ranging investigation, Study 201 (n=161 patients, 40/treatment), is regarded as supportive in nature rather than scientifically conclusive concerning the effect of E2020 on global evaluation scores, because no statistically significant overall treatment effects were seen for any measure of global improvement. An examination of the results for these measures does, however, suggest that the 5mg/day treatment group exhibited a trend toward superiority relative to placebo on the CGIC, ADL, CDR-SB and QOL measures at a number of assessment periods and at Endpoint.

In summary, two well-controlled clinical trials have provided strong evidence for the effectiveness of E2020 in the treatment of Alzheimer's Disease. The drug produces its effects by improving both cognitive status and global functioning. The improvement in both parameters exceeded standard statistical criteria. A third well-controlled clinical trial provided additional supportive evidence for the effectiveness of E2020 in the treatment of Alzheimer's Disease.

Reviewer's conclusions:

From recommendations of the PCNS advisory committee and experts in the field of AD, demonstration of a symptomatic effect in AD requires that a sponsor conduct placebo controlled, parallel, randomized, double blind trials of 3 and preferably 6 months in duration to demonstrate that the drug not only produce a significant improvement in cognitive function on a performance based test such as the ADAS-COG but that it also leads to a clinically detectable improvement. The results presented in this NDA demonstrates that the sponsor has accomplished these tasks. In two adequate and well controlled clinical trials, studies 301 and 302, the sponsor has shown that patients receiving e2020 have a statistically significant mean improvement in cognitive performance on the ADAS-COG and have a statistically significant mean clinical improvement as demonstrated on the CIBIC plus.

As seen with tacrine, another cholinesterase inhibitor, the size of the effect is modest. At 12 weeks, there appears to be a mean 2 to 3 point improvement in the ADAS-COG and 0.2 to 0.4 point improvement in the CIBIC plus. This treatment effect appears to persist with at least 24 weeks of treatment. The onset of improvement was first noted at 3 weeks, the first observation point, in study 201

and 301. In study 302, the groups were similar at 6 weeks, the first observation point, but different at the 12 weeks, the second observation point. The drug does not appear to have an effect on the underlying progression of the disease. This is suggested by the loss of cognitive function 3 to 6 week after discontinuing e2020 to a level seen in patients who were treated with placebo. The sponsor was unable to demonstrate a consistent effect in measures claiming to evaluate quality of life.

The sponsor also demonstrated that the treatment effect is dose related. In study 201, the cognitive performance of patients on doses of 1 and 3 mg/day were not significantly different from placebo whereas patients on 5 mg/day had a statistically significant improvement in ADAS-COG scores. While patients on 10 mg/day generally had better scores than patients on 5 mg/day for the ADAS-COG and CIBIC, the differences were not statistically significant. In all cases, patients on both 5 and 10 mg/day had statistically significant improvement compared to placebo for both the ADAS-COG and CIBIC plus. The 5 mg/day group had better scored on the QOL measures compared to either the 10 mg/day group or the placebo group.

Randy Levin, M.D.
Medical Reviewer

cc:
Original IND
HFD-120
HFD-120/Leber/Katz/Levin/Higgins
rl/July 2, 1996

REVIEW AND EVALUATION OF SAFETY DATA

NDA:	**20-690 (000)**
SPONSOR:	**Eisai**
DRUG:	**Aricept (donepezil, e2020)**
INDICATION:	**Alzheimer's disease**
MATERIAL SUBMITTED:	**NDA**
CORRESPONDENCE DATE:	**3/29/96**
DATE RECEIVED:	**3/29/96**
DATE COMPLETED:	**7/1/96**

Safety Database:

Studies:

The safety data base contained information from 32 studies conducted worldwide. In 28 studies, only deaths, serious AEs and withdrawals related to serious AEs were discussed. All of the safety information was presented for the remaining 4 studies, 201, 202, 301 and 302. In addition to the 32 studies, the sponsor had included serious AEs and lab abnormalities for studies 303 and 304. These studies were ongoing at the time of the NDA cut off date. Study 303 was the open label, long term extension for patients in study 301 and 302. As of 9/30/95, the date of the lab cut off, 739 patients were enrolled at doses up to 10 mg/day. Study 304 was a 6 month controlled trial conducted outside the US with a design similar to study 302. As of 12/31/95, the day of the lab cut off, 438 of a projected 750 patients had been enrolled.

Patient populations:

The sponsor divided the safety population into three groups: all patients, controlled, completed studies (201, 202, 301, 302) and uncontrolled studies (202). 13 patients enrolled at the Hartford site in study 301 were excluded. This site was discontinued due to irregularities in record keeping procedures, protocol violations and general lack of acceptable performance. Problems included missing documentation, postdated CRF information, possible forgery, failure to maintain control over the double blind study labels and incomplete maintenance of study records.

Doses:

In studies 301 and 302, patients received either 5 or 10 mg every evening. Patients on 10 mg were given 5 mg for the first 7 days in an attempt to avoid cholinergic side effects. In study 303, 5 and 10 mg given once daily were also

evaluated. In study 202, the dosing schedule was amended 4 times. The final amendment allowed patients to receive treatment up to 10 mg. In this study, patients were initially started on 3 mg/day with the dose increased by 2 mg/day up to a total dose of 7 mg/day. The dose could then be increased to 10 mg/day or decreased to as low as 3 mg/day. In study 303, patients received either 5 or 10 mg given once daily. In this study, the dose could be increased to 10 mg or decreased to 5 mg daily.

Safety evaluations:

In the sponsor's table 1B from the ISS, the safety assessments were outlined. In study 202, safety assessments took place every 3 weeks for the first 12 weeks and then every 12 weeks.

Table 1B

Schedule of Assessments for Controlled Studies in the Integrated Database

Week	0[a]	1[b]	3	6	9	12	14	15	18	21	24	30
E2020 Treatment												
E2020-A001-201		X	—	—	—	X						
E2020-A001-301		X	—	—	—	X						
E2020-A001-302		X	—	—	—	—	—	—	—	—	—	X
Treatment Washout												
E2020-A001-201						X	—	X				
E2020-A001-301						X	—	—	X			
E2020-A001-302											X	— X
Physical Examination												
E2020-A001-201	X[c]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]					
E2020-A001-301	X[c]	X[e]	X[e]	X[e]	X[e]	X[e]		X[e]				
E2020-A001-302	X[c]	X[e]	X[e]	X[e]		X[e]			X[e]		X[e]	X[e]
Vital Signs												
E2020-A001-201	X	X	X	X	X	X						
E2020-A001-301	X[c]	X[e]	X[e]	X[e]	X[e]	X[e]						
E2020-A001-302	X[c]	X[e]	X[e]	X[e]		X[e]			X[e]		X[e]	X[e]
ECG												
E2020-A001-201	X					X						
E2020-A001-301	X					X		X[f]				
E2020-A001-302	X										X	X[f]
Laboratory Determinations[g]												
E2020-A001-201	X	X	X	X	X	X	X					
E2020-A001-301	X	X	X	X	X	X		X				
E2020-A001-302	X	X	X	X		X			X		X	X
Analytical Sample[i] Collection												
E2020-A001-201	X	X	X	X	X	X	X					
E2020-A001-301		X	X	X	X	X		X				
E2020-A001-302		X	X	X		X			X		X	X
Adverse Event Monitoring												
E2020-A001-201	X	X	X	X	X	X	X					
E2020-A001-301		X	X	X	X	X		X				
E2020-A001-302		X	X	X		X			X		X	X

[a] Screening
[b] Baseline, prior to initial dose
[c] Complete: General well-being, BP, heart rate, weight, height, temperature, respiration
[e] Routine: General well-being
[f] Included chemistry, hematology, and urinalysis tests.
[g] Blood samples for TDM and the AChE
[i] If not done at last visit

Exposure:

Placebo controlled studies:

A total of 1102 patients were treated in the controlled clinical trials 201,301 and

302. 747 were treated with e2020 and 355 were treated with placebo. A fourth placebo controlled study, 304, was ongoing and still blinded at the time of the NDA submission and no exposure data was available. The distribution of patients in the three studies is summarized in the following table:

Treatment assignment for studies 201, 301 and 302				
	Placebo	5 mg	10 mg	All doses
Study 201	40	39	0	161[a]
Study 301	153	157	158	468
Study 302	162	154	157	473
Total	355	350	315	1102

[a] 42 patients were treated with 1 mg and 40 were treated with 3 mg

The exposure by dose and duration for study 301 is summarized in the following table. The duration of the study was 12 weeks (84 days). Patients in study 301 were subsequently eligible for study 303 for continued dosing.

Study 301: Exposure by dose and duration		
	Any exposure	> 3 months (≥ 80 days)
All doses	315	256
5 mg	157	136
10 mg	158	120

The exposure by dose and duration for study 302, a 24 week study, is summarized in the following table. Patients enrolled into this study were eligible to enroll into study 303.

Study 302: Exposure by dose and duration 302			
	Any exposure	> 3 months	6 months (≥160 days)
All doses	311	249	234
5 mg	154	138	127
10 mg	157	111	107

Long term extension studies:

133 of the 162 patients from study 201 were subsequently enrolled into study 202, the long term extension. The distribution of patients from each treatment group enrolling is summarized in the following table:

Enrollment of patients from study 201 into study 202				
	Placebo	1 mg	3 mg	5 mg
Study 201	40	42	40	39
Study 202	31	34	37	31

For patients involved in study 201 and 202, the duration of exposure to any dose of e2020 is summarized in the following table:

Study 201 and 202: Exposure by dose and duration					
	Any exposure	>3 months	> 6 months	> 12 months	> 24 months
All doses	161	120	103	58	10
≥ 5 mg	135	109	96	50	7
10 mg	62	9	0	0	0

Study 303 is the long term extension study of studies 301 and 302. In this study, the drug could be increased to 10 mg/day or decreased to 5 mg/day at any visit before week 72. 384 patients from study 301 and 356 patients from study 302 were enrolled into study 303. The sponsor noted that 196 patients completed ≥ 50 weeks of treatment by 9/31/95. The distribution of the patients enrolling into study 303 is summarized in the following table:

Enrollment of patients in study 301 and 302 into study 303				
	Patients in study 301	Entering study 303	Patients in study 302	Entering study 303
Placebo	153	133	162	128
5 mg	157	130	154	128
10 mg	158	121	157	100
Total	468	384	473	356

Total exposure by dose and duration:

Studies 201, 202, 301 and 302:

All safety data from studies 201, 202, 301 and 302 were included in the safety data base. The number of patients exposure to recommended doses of e2020 in these studies and the duration of the exposure is summarized in the following table.

Number of patients exposed to E2020 by dose and duration (studies 201, 202, 301 and 302)

	All doses			5 mg			10 mg		
Exposure	Any	> 3 months	> 6 months	Any	> 3 months	> 6 months	Any	> 3 months	> 6 months
201/202[a]	152	120	103	135	109	96	62	9	0
301	315	256	0	161	136	0	163	120	0
302	311	249	234	154	138	127	157	111	107
All studies	778	625	337	450	383	223	382	240	107

[a] in study 202 patients could be on both 5 and 10 mg/day. Patients on 7 mg were included in the 5 mg/day group.

Only 50 patients have been exposed to doses of 5 mg for 1 year or more. No patients were exposed to doses of 10 mg for 1 year or more.

Study 303:

Serious AEs and lab abnormalities from study 303, the long term extension study for studies 301 and 302, were included in the safety data base. The cut off date for reporting serious AEs was 6/30/95 and for lab data was 9/31/95. Taking into account patients enrolled into study 303, an additional 261 patients exposed to placebo in study 301 and 302 were exposed to doses of ≥ 5 mg and 479 patients already exposed to doses of 5 or 10 mg/day for 3 to 6 months were continued on e2020. The sponsor noted that 196 patients had completed ≥ 50 weeks of treatment. From the information in the data base, at least 300 of patients were exposed for ≥ 24 weeks. 95% of the patients in study 303 were titrated to 10 mg/day.

Demographics (studies 201, 301, 302):

For the placebo controlled trials, the mean age of patients was 73, ranging from

50 to 94. 5% of the patients were ≥ 85 and 15% were < 65. 95% of the population were white and 63% were female. The demographics were similar between groups and was similar to the patients entering study 202.

Baseline characteristics (studies 201, 301, 302):

76% of all patients had a CDR rating of 1. The mean MMSE score was 19 with scores ranging from 8 to 27. The treatment groups were similar. Other baseline characteristics including medical history, prior medication, concomitant medication, height and weight were similar between groups.

Disposition (studies 201, 301, 302):

75% or more of all patients completed the controlled clinical trials. Less than 4% discontinued for a serious AE. The disposition of patients is summarized in the following table:

Disposition of patients in the controlled clinical trials						
	Number (%) of patients					
	Placebo	1 mg/day	3 mg/day	5 mg/day	10 mg/day	All E2020
Total	355	42	40	350	315	747
Number of Patients who Completed	307 (86)	34 (81)	38 (95)	306 (87)	235 (75)	613 (82)
Number of Patients who Discontinued	48 (14)	8 (19)	2 (5)	44 (13)	80 (25)	134 (18)
Reason for Discontinuation:						
Adverse Event	16 (5)	5 (12)	2 (5)	19 (5)	42 (13)	68 (9)
Request of Patient/Investigator	8 (2)	1 (2)	0	8 (2)	11 (3)	20 (3)
Medication Noncompliance	1 (<1)	0	0	2 (1)	5 (2)	7 (1)
Protocol Violation	12 (3)	1 (2)	0	6 (2)	11 (3)	18 (2)
Other	11 (3)	1 (2)	0	9 (3)	11 (3)	21 (3)

Of the 141 patients who completed study 201, 133 entered study 202. 42 (32%) continued in the study at the time of the cut off date. 76 (57%) discontinued. 14 (11%) discontinued for an AE, 9 of which were serious. 26 (20%) by request of the patient or investigator and 28 (21%) discontinued because of lack of efficacy or withdrawal of consent.

Deaths:

There were 27 deaths that occurred either during the studies or within 4 weeks of discontinuing in all of the studies.

There were 4 deaths during the controlled clinical trials, 201, 301 and 302. Three deaths occurred in patients assigned to placebo (N=355) and 1 assigned to 10 mg/day (N=315). The patient in the 10 mg/day group died from metastatic disease.

There were 5 deaths in study 304, a 6 month, double blind efficacy study. 739 patients were enrolled in this study with 488 assigned to active treatment. Since this efficacy study was ongoing at the time of the submission, the data was blinded.

One patient involved in study J081-134, a double blind study involving 190 patients randomized to 0, 3 or 5 mg/day for up to 16 weeks of treatment died. This patient had a stroke after 32 days of treatment with 5 mg/day.

17 deaths were reported in the long term extension studies, 202, 303 and 305. Three patients were in study 202 (131 patients exposed), 9 were in study 303 (767 patients exposed, 196 for $\geq$ 50 weeks) and 4 were in study 305 (number unknown). The sponsor's summary of the deaths are in table 7A. Narratives of the patients who died in all studies is in Appendix 1 of this review.

Table 7A

Deaths

Protocol E2020-	Patient	Treatment	Days of Treatment Received[1]	Investigator-Assessed Relationship to Test Drug	Cause of Death

Table 7A (Cont'd)

Deaths

Protocol [illegible]	Patient	Treatment	Days of Treatment Received[1]	Investigator-Assessed Relationship to Test Drug	Cause of Death

Discontinuations for adverse events in controlled clinical trials:

In studies 201, 301 and 302, 5% of patients on placebo and 5% of patients on 5 mg/day withdrew for AEs. 13% of patients on 10 mg/day withdrew for AEs. Out of the 68 patients withdrawing for AEs, 52 of the patients withdrew for non serious AEs, mostly for nausea, diarrhea and vomiting. Other non serious AEs included dizziness, depression and aggression. I took all patients who withdrew for AEs. If the rate was ≥ 1% in either the 5 or 10 mg group and was greater than the rate observed in the placebo group, I included it in the following table.

Studies 201, 301 and 302: Percentage of patients withdrawing for any AE			
AE	Placebo (N=355)	5 mg (N=350)	10 mg (N=315)
Total (serious AEs)	5 (2)	5 (2)	13 (3)
Accident	<1	0	1
Fatigue	0	0	1
Nausea	1	1	3
Diarrhea	0	<1	3
Vomiting	<1	<1	2
Abdominal disturbance	<1	0	<1
Anorexia	<1	1	0
Weight decrease	0	0	1
Fracture Bone	0	0	1
Depression	0	1	1
Dizziness	0	1	<1
Aggression	0	1	1
Insomnia	<1	0	1

Discontinuations for adverse events in extended clinical trials:

In study 202, 14 of 133 patients (11%) withdrew for adverse events. The causes of discontinuation were GI complaints in three patients (stomach pain, heartburn, GI bleeding from ulcers), stroke in two patients, and in single patients; hallucinations, agitation, headache, fall, syncopal spell, UTI, depression and seizure.

In study 303, over 700 patients were enrolled from study 301 and 302 with 196 patients exposed to e2020 for ≥ 50 weeks (95% at doses of 10 mg/day). 12 patients discontinued for serious AEs. 7 of these patients had syncopal episodes. This compares to 1 in 133 patients in study 202 (0.8%). The onset of syncope ranged from 7 to 31 weeks after initiation of treatment. Other events included renal failure, gastric ulcer, epigastric pain, seizure and agitation.

Narratives for patients discontinuing for serious AEs:

A narrative for all patients discontinuing for serious AEs in the safety data base is in Appendix 2. This includes information from patients in studies 201, 301 and 302 as well as from studies 202, 303 and 304.

Other Serious AEs in the controlled clinical trials:

In the controlled clinical trials, 47 of the 747 patients on drug (6%) and 18 of the 355 patients on placebo (5%) had at least one serious AE. The percentage of serious AEs noted in ≥ 1% of patients on 5 or 10 mg/day with a higher rate than in patients on placebo is summarized in the following table.

Percentage of patients with a serious AEs in the controlled clinical trials			
AE	Placebo (N=355)	5 mg (N= 350)	10 mg (N=315)
At least one SAE	5	5	7
Accident	0	1	2
Chest pain	<1	1	<1
pain	0	0	1
hernia	0	0	1
infection	0	2	0
syncope	<1	1	<1
Angina	0	1	0
Fracture bone	0	0	2
aphasia	0	0	1

Serious AEs in study 202:

34 of the 131 patients (26%) in study 202, had at least one SAE prior to the cut off date of 1/30/95. Serious AEs (number of patients) included cancer (8) (squamous cell carcinoma, prostatic (3), basal cell, transitional cell, breast, uterine), agitation (5), syncopal spells (2), stroke (2), seizure (2), fall (2), low back pain (2). Other serious AEs that occurred in only one patient included GI bleed related to duodenal ulcer, UTI, hallucinations, depression, death (MI (1)), unknown, angina, bronchitis, renal colic, atrial arrhythmia.

Serious AEs in study 303:

In study 303, 67 of the 739 patients exposed to the drug, 196 of whom were treated for ≥ 50 weeks, had at least one serious AE (including death) as the cut off date of 6/30/95. The serious AEs (number of patients) included syncope (10), cancer (8), surgery (7), fall/fracture (6), UTI (5), pneumonia (4), atrial fibrillation (3), intracerebral bleed (3) and MI (3), stroke (2), vomiting (2), bronchitis (2) . Other serious AEs occurring in only one patient included pulmonary embolism, viral encephalitis, hemoptysis, renal failure, abdominal pain, depression, sepsis, retinal vein thrombosis, agitation, cholecystitis, bleeding ulcer, agitation.

Treatment Emergent Adverse events:

Methods:

I took the double blind portion of studies 201, 301 and 302 and found all of the adverse event terms for the placebo, 5 and 10 mg treatment groups. The frequencies of AEs were calculated by dividing the total number of AEs by the number of patients exposed to the drug; 355, 352 and 318 for the placebo, 5 and 10 mg groups, respectively. I combined the 5 and 10 mg group and took all of the AEs that were more frequent in the e2020 group when compared to placebo, eliminated those with a frequency < 1% for the combined group and placed the remainder in the table below:

Studies 201, 301 and 302: Adverse events							
	Plb (N=355)		5 mg (N=352)		10 mg (N=318)		5+10 mg (N=670)
	N	%	N	%	N	%	%
Nausea	19	5.35	20	5.68	59	18.55	11.79
Diarrhea	14	3.94	24	6.82	45	14.15	10.30
Insomnia	18	5.07	19	5.40	41	12.89	8.96
Headache	28	7.89	27	7.67	32	10.06	8.81
Pain	26	7.32	28	7.95	29	9.12	8.51
Dizziness	19	5.35	28	7.95	25	7.86	7.91
Accident	18	5.07	25	7.10	19	5.97	6.57
Muscle Cramp	6	1.69	19	5.40	24	7.55	6.42
Fatigue	11	3.10	13	3.69	24	7.55	5.52
Vomiting	9	2.54	10	2.84	25	7.86	5.22
Anorexia	5	1.41	9	2.56	18	5.66	4.03
Rhinitis	9	2.54	11	3.12	13	4.09	3.58
Infection	6	1.69	13	3.69	10	3.14	3.43
Stomach Upset	6	1.69	13	3.69	9	2.83	3.28
Dreams Abnormal	0	0.00	10	2.84	8	2.52	2.69
Ecchymosis	7	1 97	11	3.12	7	2.20	2.69
Confusion	8	2.25	7	1.99	10	3.14	2.54
Depression	2	0.56	7	1.99	10	3.14	2.54
Weight Decrease	4	1.13	5	1.42	12	3.77	2.54
Upper Resp Tract Infection	8	2.25	10	2.84	6	1.89	2.39
Anxiety	4	1.13	5	1.42	9	2.83	2.09

Studies 201, 301 and 302: Adverse events (continued)							
	Plb (N=355)		5 mg (N=352)		10 mg (N=318)		5+10 mg (N=670)
	N	%	N	%	N	%	%
Arthritis	3	0.85	3	0.85	9	2.83	1.79
Frequent Urination	2	0.56	6	1.70	6	1.89	1.79
Somnolence	1	0.28	5	1.42	6	1.89	1.64
Syncope	3	0.85	9	2.56	2	0.63	1.64
Weakness Generalized	1	0.28	7	1.99	4	1.26	1.64
Aggression	0	0.00	2	0.57	7	2.20	1.34
Incontinence Fecal	2	0.56	1	0.28	8	2.52	1.34
Dyspnea	2	0.56	3	0.85	5	1.57	1.19
Tooth Disorder	1	0.28	4	1.14	4	1.26	1.19

Severe AEs:

The AEs rated as severe that were more frequent in the e2020 group compared with placebo included accidents, headaches, vomiting, nausea and fractured bones. All were present in 1% of patients on 10 mg/day and < 1% in the placebo group.

Occurrence of AEs by time on treatment:

The sponsor conducted an analysis of the incidence of AEs by exposure to the drug. The first incidence of nausea, diarrhea, vomiting, insomnia, fatigue and anorexia occurred within the first 3 weeks of treatment in more than 50% of patients. Muscle cramps occurred more frequently during weeks 4 to 12.

AEs during the placebo washout period:

No new events occurred in more than 2% of the patients receiving 10 mg/day during the washout period.

AEs during the open label extension (study 202):

All AEs occurring at a frequency of > 5% during the open label extension, study 202, are summarized in the following table:

Study 202: Percentage of patients with AEs		
AE	Overall incidence	Severe
Agitation	18	2
UTI	10	1
URI	8	0
Confusion	8	1
insomnia	7	1
dizziness	7	0
Fracture bone	5	2
anxiety	5	1
coughing	5	0
depression	5	1
vertigo	5	0
hallucination	5	2

Vital signs:

For the controlled clinical trials, in the 10 mg group, there was an overall increase in pulse of 1.9 bpm, a decrease in systolic BP of 3.1 mmHg and a decrease in diastolic BP of 2.7 mmHg. Clinically significant changes occurred in 5 placebo patients, 5 patients on 5 mg/day and 6 patients on 10 mg/day. The changes are summarized in the following table:

Number of patients with clinically significant changes in BP and/or heart rate (HR) (range of changes)			
Change	Placebo (N=355)	5 mg/day (N=352)	10 mg/day (N=315)
Decrease in HR	1 (48 bpm)	1 (48 bpm)	0
Increase in HR	1 (136 bpm)	1 (127 bpm)	0
Decrease in BP	0	1 (systolic to 82)	1 (diastolic to 49)
Increase in BP	3 (systolic to 181 - 220)	4 (systolic to 184 - 186, diastolic to 114)	5 (systolic to 184 - 208, diastolic to 108)

In the open label study, 202, there was one patient on e2020 who had a clinically significant change in vital signs. Patient 069 had a BP of 198/106 at week 48 while on 5 mg/day. At week 84, the diastolic BP was 110. During the washout period, the patient was noted to have labile BP measurements.

ECGs:

Investigators categorized the ECGs as normal, abnormal but not clinically significant and abnormal and clinically significant. The number of patients with a change in ECG from normal at baseline to clinically significantly abnormal was as follows: placebo 2/298 (1%), 1 mg/day 0/37 (0%), 3 mg/day 2/37 (5%), 5 mg/day 5/291 (2%), 10 mg/day 0/222 (0%). The changes are summarized in the following table:

ECG changes rated as clinically significant[1], serious[2], and/or leading to withdrawal[3]		
Dose	Patient ID	ECG abnormality
Placebo (N=299)	301-135	Atrial fibrillation2
	301-164	LBBB[1]
	301-308	Sinus bradycardia[1], PVCs[1]
1 mg (N=37)	201-30	ECG abnormal[3]
3 mg (N=37)	201-6	Atrial fibrillation[1]
	201-157	Absent p wave[1]
5 mg (N=291)	201-138	Supraventricular tachycardia[3]
	301-9	Arrhythmia with pacemaker[2]
	301-17	PVCs[1], possible RBBB[1]
	301-149	Atrial fibrillation[2]
	301-309	Sinus bradycardia[1], LAD[1], increased QRS voltage[1]
	302-2	Frequent PVCs[1], multifocal PVCs[1], atrial fibrillation[1,3]
	302-31	Sinus arrhythmia[1], minor IVCD[1], t wave inversions[1]
	302-273	Multifocal PVCs[2,3]
	302-424	Possible old MI[1], sinus bradycardia[1]

The only ECG change reported as possibly dose related was sinus bradycardia. For all reports of sinus bradycardia, the incidence was as follows: placebo 4/299 (1.3%), 1 mg/day 1/37 (2.7%), 3 mg/day 1/37 (2.7%), 5 mg/day 26/291 (8.9%), 10 mg/day (9.5%).

Labs:

Methods:

The sponsor reported (1) the change in clinical laboratory values from baseline to the last value on treatment and (2) any value that was qualified as an abnormal value based on set criteria provided by the division. Abnormal values obtained during the placebo washout were also included.

Mean changes in lab values:

The mean changes and number of patients with clinically relevant shifts were similar in the placebo and drug groups. The mean lab values increased in the 5 and 10 mg group relative to placebo for ALT, AST, LDH and CPK measurements. The mean glucose level decreased in the 5 and 10 mg group compared to placebo. A large shift in CPK in the 5 mg group was related to a single patients who had elevations associated with long distance cycling. The changes are summarized in the following table:

Study 201, 301 and 302: Mean change in lab values during the controlled clinical trials			
Lab value	Placebo	5 mg	10 mg
ALT	0.32	2.09	2.41
AST	0.25	1.26	2.11
LDH	1.3	2.93	2.08
CPK	-0.21	10.63	1.95
Glucose	0.38	-2.55	-2.59

Shift in lab values from normal to abnormal:

There were no lab value where the frequency of patients with a shift from normal to abnormal was greater than 3% in the 10 mg group compared to placebo. There were no changes noted during the placebo phase washout.

During the long term extension study, 202, there were few patients with clinically relevant changes from baseline to endpoint. 4% of patients had a high BUN though none were associated with a creatinine above 2.0. 5% of patients had an elevation of the CPK. 7% had an elevation of glucose and 5% had a reduction in glucose. 14% had low calcium but the lowest level was 8.0. In the urine analysis, 18% had occult blood in the urine, 11% had glucose in the urine and 5% had protein in the urine. Overall, the changes were not thought to be clinically significant.

Lab values for studies 303 and 304:

Lab data was collected through 9/31/95 for study 303 and 12/31/95 for study 304. The greatest number of lab abnormalities determined to be AEs included elevated BUN at 5%, elevated glucose at 6%, low calcium at 3%, elevated CPK at 4%, and

a decrease hematocrit at 6%. In most patients, the lab abnormalities did not change or fluctuated over the course of the study.

LFTs:

Two patients withdrew for elevated LFTs.

Patient 105 in study 201 was randomized to 3 mg/day and at day 75 was noted to have an increase in ALT to 75 which peaked at 163 on day 82. The drug was discontinued and the ALT fell to 52 by day 96. Patient 041 in study 301 was on 10 mg/day. The ALT level increased to 508 on day 76. The drug was discontinued and the ALT fell to 69 on day 81 and was 15 on day 97.

Renal function tests:

The shifts from normal to abnormal were mixed between placebo and drug. There did not appear to be a dose related effect on renal function.

CPK:

Patients on drug had a higher mean CPK then patients on placebo. I reviewed the patients in study 302 with an elevation of the CPK (17 on placebo, 28 on 5 mg/day and 31 on 10 mg/day). The elevations did not appear to be associated with symptoms. 3 of the placebo patients had evidence of injury (ecchymosis or [illegible]dents reported) that [illegible]uld possibly explain the change in CPK. Compiaints of fatigue, weakness, muscle cramping were not associated with the elevations in the CPK. In study 303, patients with CPK elevations had fluctuating levels during the course of the study.

Safety information from other sources:

The sponsor conducted 6 phase 1 and 6 phase 2 studies in Japan. 357 patients received doses between 0.1 and 5 mg in phase 2 trials. 82 had received doses of 5 mg. The sponsor reported that the safety profile was similar to that seen in the controlled clinical trials.

Phase 1 studies in the US involved over 700 patients. The sponsor did not report any serious adverse events.

Drug-Drug interactions:

The sponsor formally conducted interaction studies with digoxin, theophylline, cimetadine and warfarin. See the PK review for additional information.

Drug-Demographics interaction:

There did not appear to be a significant difference between groups with the common adverse events based on the age, sex of the patients though nausea and vomiting appeared to be more prominent in the female patients compared to the male patients.

Drug-disease interactions:

There did not appear to be a significant difference in the PK in patients with renal or hepatic impairment.

Sponsor's conclusions:

E2020 is a piperidine-based, reversible, acetylcholinesterase inhibitor that specifically inhibits brain acetylcholinesterase, resulting in an increase in brain acetylcholine. Results of animal studies and human studies demonstrate that, unlike the acridine-based inhibitors, E2020 is devoid of hepatotoxicity. Results presented in this ISS demonstrated that E2020 doses up to 10 mg/day did not affect liver function tests during the 12 or 24 week treatment periods of the controlled studies or during the one year of treatment in the ongoing, long-term extension studies.

E2020 was well-tolerated by the elderly, Alzheimer's Disease patients in these studies. While greater proportions of patients given 10 mg/day E2020 relative to those given 5 mg/day or placebo withdrew from treatment, withdrawals due to adverse events in this group were infrequent (13%). The most common adverse events that led to withdrawal in the 10 mg/day group were nausea, diarrhea, and vomiting. However, the proportion of patients in the 10 mg/day group who withdrew because of these SS were only 3.5%, 3.2, and 1.6%, respectively. Thus the 10 mg/day dose of E2020 was well-tolerated by the great majority of patients.

Greater proportions of patients given 10 mg/day E2020 relative to those given placebo experienced nausea, diarrhea, vomiting, fatigue, muscle cramp, insomnia, and anorexia. Two of these events, diarrhea and muscle cramp, showed a relationship to the 5 mg/day E2020 dose as well. For the small minority of patients experiencing these SS, episodes were transient and resolved on continued E2020 treatment.

For the 10 mg/day group, the Digestive System TESS experienced were mild or moderate in severity, occurred predominantly during the first 3 weeks of treatment, typically lasted 1-2 days, and are expected with compounds that increase cholinergic activity.

Incidences of nausea, diarrhea, vomiting, fatigue, insomnia, and anorexia generally increased with both E2020 dose and age and were experienced by a greater proportion of the female population relative to the male population. The incidence of muscle cramp was higher in the female population given 10 mg/day E2020 relative to the male population given 10 mg/day but was independent of age.

Overall, the elderly population in these studies tolerated E2020 treatment well and TESS related to treatment were mild or moderate and abated during continued treatment.

Safety conclusions:

The drug appears to be relatively well tolerated. Over 75% of patients in the highest dose group of 10 mg/day were able to complete the 3 to 6 month studies. Of the adverse events that occurred, 95% were rated as mild to moderate in severity. The most common problems with the drug were GI related, specifically, nausea, vomiting and diarrhea which is consistent with the expected cholinergic effects.

Some safety concerns for the cholinergic drugs such as GI ulcers, bradycardia, etc have been seen with this drug in small numbers of patients. While the incidence of these events do not suggest a causal relationship, precautions concerning the events seen for the drug class should be included in labeling.

Potential problems seen in the data base included falling and syncope. These were more common serious AEs though the cause is not known. An elevation of the CPK was also seen without clear cause or clinical significance.

In describing the safety of this drug, one drawback is the lack of long term experience, especially at the highest recommended dose of 10 mg/day. The NDA only contained complete safety information from 133 phase 2 patients treated long term with the drug. Since the maximum dose of the drug until late in phase 2 was 5 mg/day, there was essentially no experience at doses of 10 mg/day. The sponsor supplemented the safety data base with information on serious AEs and lab abnormalities in patients involved in the phase 3 long term extension studies, 303 and 305. In these studies, patients were allowed to be titrated to doses of 10 mg/day. In study 303, 95% of the patients were titrated to 10 mg/day. At the time of the cut off to the NDA, 196 patients were treated for about one year. Overall, in this long term experience, the pattern of serious AEs support the relative safety of this drug in patients with AD.

Randy Levin, M.D.
Medical Reviewer

cc:
Original IND
HFD-120
HFD-120/Leber/Katz/Levin/Higgins
rl/July 2, 1996

Appendix 1: Narratives of patients who died during studies:

Placebo:

Study 201, patient 012: This was a 79 year old female who completed the 12 weeks of treatment on placebo. Following discontinuation of the drug, the patients had an increase in weakness and **refused intake**. She died in her home almost 3 weeks later.

Study 301, patient 320: This was an 83 year old male who was on placebo for about 9 weeks when he was admitted for hematemasis which was diagnosed as being related to a **gastric ulcer**. The study treatment was discontinued. The patient was transferred to a nursing home and died about 2 weeks later from **renal failure**.

Study 302, patient 355: This was a 73 year old female who was on placebo for 22 days when she complained of chest pressure. She was hospitalized and the next day complained of severe chest pain and died. A **pulmonary embolism** was suspected as the cause of death.

5 to 7 mg:

Study 202, patient 134: This was a 69 year old male with a total exposure of 340 days. The patient was titrated to a dose of 7 mg/day. The drug was discontinued because the caregiver thought that the drug was longer providing a benefit. One day after discontinuation, the patient was found to be in atrial fibrillation. The patient was admitted to the hospital and subsequently deteriorated cognitively. The patient was then discharged to a long term care facility. Approximately 3 weeks following discontinuation of the study drug, the patient developed a fever to 104.6. The patient subsequently died and the cause of death was cardiorespiratory arrest due to **aspiration**.

Study 202, patient 147: This was a 68 year old female who was on 7 mg/day. The patient was a passenger in a car involved in a motor vehicle **accident**. She died at the site of the accident.

Study 303, patient 360-302. This was an 81 year old female who received 5 mg/day for 22 weeks. After treatment for 4 weeks, the patient had a **stroke**. She continued on treatment. After 18 weeks, she collapsed at home and subsequently died. A recurrent stroke was the diagnosis as the cause of death.

Study 303, patient 378-302. This was a 70-year-old female who received open-label E2020 5 mg daily for 5 weeks at the time of her death. The patient

related to her caregiver that she had severe cold symptoms (cough, pharyngitis, rhinitis) and wanted to go to bed early. She was found dead in her room about 8:00 p.m. by the caregiver who went in to give the patient her medications. An autopsy revealed severe acute retroperitoneal hemorrhagic **pancreatitis due to cholelithiasis.**

Study J081-134, patient 203/ This was a 73 year old male who was on 4 weeks of study treatment. The patient's level of consciousness decreased and a work up found an arrhythmia and subsequently, the patient had a **cardiac arrest.** The patient was also on distimgime bromide.

10 mg:

Study 202, patient 068: This was a 69 year old male who was titrated to 10 mg/day after being on 7 mg/day for 261 days. The dose was reduced to 7 mg/day after the patient had diarrhea for 2 days. The drug was subsequently discontinued because of heartburn. 9 days of the drug was discontinued, the patient **collapsed** and was unable to be resuscitated. Total exposure was 329 days.

Study 302, patient 326: This was an 80 year old female who was titrated to 10 mg/day. She was on treatment for about 10 weeks when she was discontinued from treatment when she developed was diagnosed with a **DVT** and placed on coumadin. The patient died 1 month after the study treatment was discontinued.

Study 303, patient 070: This was a 58 year old female who was enrolled in the open label study and received 5 mg/day for 29 weeks followed by 10 mg/day for an additional 33 weeks. She developed severe vomiting and was diagnosed with **pancreatitis**. The patient had gallstones removed endoscopically. She went into septic shock and died 5 days after discontinuation of the study treatment.

Study 303, patient 131-301. This was a 83 year old female. She received 5 mg for 6 weeks and 10 mg for an additional 39 weeks. The patient was diagnosed with leiomyosarcoma. The investigator felt that the tumor predated the patient's participation in the study. The patient died of an **aspiration pneumonia.**

Study 303, patient 15-301/ This was a 78 year old male who received 5 mg/day for 6 weeks and then 10 mg/day for 2 weeks. The patient developed severe **chest pain** and collapsed and was pronounced dead on arrival to the hospital.

Study 303, patient 187-302/ This was a 74 year old female who was on 5 mg/day for 6 weeks and then 10 mg/day for an additional 18 weeks. She developed chest pain and after transfer to the hospital and developed **cardiac**

arrest and subsequently died.

Study 303, patient 346-301. This was an 82 year old female who was 5 mg/day for 6 weeks and then 10 mg for 2 weeks. She was known to have transitional cell carcinoma of the bladder at the time of enrollment. She developed obstructive **renal failure** and consequently died.

Study 303, patient 382-301. This was a 75 year old female who was treated with 5 mg/day for 6 weeks and then 10 mg/day for an additional 20 weeks. The patient collapsed at home and was found to have a massive right sided **parietal lobe hemorrhage**. She subsequently died.

Study 305, patient 254-304 : This was an 82 year old male who received 5 mg/day for 6 weeks and then 10 mg/day for an additional 14 weeks. The patient was admitted with a **pneumonia** and the drug was discontinued the next day. The patient died 5 days later.

Study 305, patient 349-304. This was a 79 year old male who was on 5 mg/day for 6 weeks and then 10 mg for 9 weeks. The patient was diagnosed with **pneumonia** and heart failure. His condition worsened and the patient subsequently died.

Study 305, patient 391-304. This was a 73 year old male who was on 5 mg/day for 6 weeks and then on 10 mg/day for 11 days when he complained of chest pain. The patient died and the diagnosis was a **myocardial infarction**.

Blinded treatment:

Study 304, patient 069. This was a 77 year old female who was on double blind treatment for 18 weeks when she was admitted to the hospital with an **aspiration pneumonia**. She was continued on treatment and approximately 7 weeks later she died.

Study 304, patient 205. This was a 74 year old female who was on double blind treatment for almost 22 weeks. She had complained of indigestion and was found dead the next day. An autopsy confirmed that the patient had an **myocardial infarction** from a thrombus in the left anterior descending artery.

Study 304, patient 442. This was a 60 year old female who was on double blind treatment for 7 weeks when she noted shortness of breath and chest pain. 4 days later, she was admitted to the hospital and diagnosed with a **pulmonary embolism**. She died later the same day.

Study 304, patient 452 This was a 70 year old female who received double blind treatment for 5 days when she hit by a car and died from her injuries.

Appendix 2: Narratives for patients discontinuing for serious AEs:

Placebo:

Study 201, patient 136: This was a 58 year old female on placebo who was discontinued for restlessness and **agitation.**

Study 301, patient 320: This was an 83 year old female who was randomized to placebo. She was on placebo for 9 weeks when she developed hematemasis from a **gastric ulcer.**

Study 302, patient 076: This was a 59 year old male who was on placebo for 70 days when he was noted to have **ataxic gait,** drooling, slurred speech and a headache and was hospitalized and the study medication was discontinued.

Study 302, patient 161: This was an 88 year old female who was randomized to placebo. After 28 weeks of treatment, the patients had a **stroke.** The patient's symptoms were nonspecific. The patient was not eating, and would not walk.

Study 302, patient 292: This was a 63 year old male who was on placebo for about 15 weeks when the patient experienced shortness of breath and diaphoresis. An MI was ruled out. 5 weeks later, the patient had SOB with loss of consciousness (**syncope**) for several seconds while taking a walk. The patient was hospitalized and diagnosed with a septal **myocardial infarction.** The patients also had evidence for bradykinesia.

Study 302, patient 399: This was a 75 year old female who was on placebo for 3 days when the study treatment was discontinued for abdominal pain which was related to a **small bowel obstruction** from a femoral hernia.

1 to 3 mg:

Study 201, patient 161: This was a 58 year old male who was on 1 mg/day. After 9 days, the patient suffered a **stroke.** Details are not available on the CRF.

Study 202, patient 090: This was an 84 year old female who was on 1 mg/day during the double blind treatment. She had GI bleeding following the double blind phase. She was started on 3 mg/day in study 202 and after 43 days was discontinued because of the **GI bleeding.** The patient was also on etodolac.

5 to 7 mg:

Study 201, patient 070: This was a 78 year old female on 5 mg/day who had a

syncopal episode 1.5 hours after her first dose. She also complained of nausea and drooling. No cause of the episode was discovered.

Study 201, patient 088: This was a 66 year old female on 5 mg/day who was noted to have hematuria prior to receiving study medication. 15 days after starting treatment she was found to have a small **bladder cancer**.

Study 201, patient 128: This was a 73 year old male on 5 mg/day who had received 20 days of treatment when she was noted to have a **tachycardia** (160 bpm) and **hypotension** (BP 80/60) on a routine visit. No CRF in the CANDA.

Study 301, patient 034: This was a 76 year old female who was randomized to 10 mg/day and received 2 days of treatment prior to **falling** on an icy driveway and breaking her tibia.

Study 302, patient 273: This was an 84 year old female who received 5 mg/day for 11 weeks when she fainted (**syncope**) while sitting in a chair. The ECG was unchanged from admission with a normal sinus rhythm with multifocal PVCs.

Study 302, patient 398: This was a 75 year old female who was on 5 mg/day for about 10 weeks when she developed nausea and weakness. She was diagnosed with **pyelonephritis** and renal failure. Urine cultures were positive. She was treated with antibiotics.

Study 302, patient 481: This was on 5 mg/day and was discontinued after 126 days after having a **total knee replacement**.

Study 202, patient 027: This was a 77 year old female who was titrated to 7 mg/day. After treatment for about 5 months, the patient was found to have a grade II **transitional cell carcinoma** which was removed.

Study 202, patient 028: This was a 56 year old female who received 3 mg/day and was titrated to 7 mg/day. She was found to have breast cancer after about 1 year of treatment. Study treatment was continued and at the week 100 visit, the patient was noted to have a decrease in blood pressure (**hypotension**).
Study 202, patient 040: This was an 85 year old female who was on 5 mg/day during study 201 was enrolled into study 202 and titrated to 7 mg/day. Two months after enrolling in study 202, the patient was noted to be agitated with hallucination and anxiety. The drug was discontinued and the patient's condition did not improve.

Study 202, patient 089: This was a 78 year old female who was titrated to 7 mg/day. After treatment for 105 days, the patient was diagnosed with

depression.

Study 202, patient 091: This was a 71 year old male who was on 5 mg/day during study 201 and was titrated to 7 mg/day in study 202. The drug was discontinued after one year when the patient's **agitation** led to placement outside the home.

Study 202, patient 127: This was an 85 year old male who was on 5 mg/day during study 201. The patient was titrated to 7 mg/day. After about 6 months of treatment, the patient had a **stroke**. The patient had a second stroke which was associated with a seizure two months later.

Study 202, patient 145: This was a 64 year old female who was on 1 mg/day in study 201. She was titrated to 7 mg/day and was on that dose for 221 days when she had a focal **seizure**. She was treated with Dilantin.

10 mg:

Study 202, patient 158: This is an 81 year old female who was titrated to a dose of 10 mg/day. She had been on treatment for 344 days when she **fell** and was hospitalized for multiple injuries including a pneumothorax.

Study 301, patient 231: This was a 75 year old female who experienced chest pain, vomiting and sweating 24 hours after receiving her first dose of 10 mg. She was diagnosed with an anterolateral **myocardial infarction**. She was a smoker and had a history of hypertension.

Study 302, patient 018: This was an 84 year old male who was randomized to 10 mg/day. After 3 months of treatment, the patient underwent surgery for a hernia repair. The day following surgery, the patient was reported to have suffered a mild subendocardial **myocardial infarction**. The patient was noted to have had a similar episode 6 months ago. The patient died 2 months after discontinuation of the study drug from a pneumonia.

Study 302, patient 081: This was a 79 year old male who was on 10 mg/day for 9 days when he became more **agitated** and **fell**.

Study 302, patient 155: This was a 75 year old male who was on 10 mg/day for 23 weeks when he was diagnosed with metastatic non small cell **carcinoma**.

Study 302, patient 275: This was an 83 year old female who was on 10 mg/day for 11 weeks when she stumbled and **fell** while using her walker and fractured her pelvis.

Study 302, patient 393: This was a 70 year old female was on 10 mg/day who was discontinued from the study after about 4 months of treatment after having **knee surgery**. The patient was thrown from a horse resulting in knee injury. The patient had also had diarrhea after being treated with antibiotic following the knee surgery.

Study 302, patient 408: This was an 81 year old female who was on 10 mg/day for 3 weeks when she had a **stroke**. No details provided.

Study 302, patient 456: This was an 82 year old male who 10 mg/day for 9 weeks when he had a **syncopal episode**. The patient had a history of sinus bradycardia.

Study 303, patient 006-301: This was a 74 year old male who was on 10 mg/day in study 301 and on 5 mg/day in study 303 for 4 days when the patient stopped urinating. An evaluation showed **renal failure** secondary to obstruction. No CRF in the CANDA. Paper CRF reviewed.

Study 303, patient 019-301: This is a 67 year old female who was on 10 mg/day in study 301 and then was on 5 mg/day for 3 weeks in study 303 when she had 2 syncopal spells. The patient was continued on drug and the dose was increased to 10 mg/day. 4 weeks after being on 10 mg/day (a total of about 8 weeks in study 303), the patient had several episodes of **syncope** or near syncope. No cause for the syncopal spells could be found.

Study 303, patient 033: This was an 84 year old female who was on 10 mg/day in study 301 and on 5 mg/day in study 303 for 6 days when she was noted to have gastric distress. She was found to have a bleeding **gastric ulcer**.

Study 303, patient 035: This is a 76 year old female who was on placebo during study 301 and was on 5 mg/day for 6 weeks and 10 mg/day for 21 weeks when she fell and subsequently had multiple syncopal spells. The spells were associated with nausea and vomiting. Her ECG showed bradycardia, intermittent first degree heart block and non specific T wave changes all of which noted pre enrollment. The diagnosis was vasovagal **syncope**. The nausea and vomiting were thought to possibly be related to an increase in a NSAID dose.

Study 303, patient 037: This is a 79 year old female on 5 mg/day in study 301 and enrolled into study 303 with an increase in dose to 10 mg over 7 weeks had a **syncopal spell**. The CRF could not be found on the CANDA.

Study 303, patient 122: This was a 77 year old female who was on placebo in study 302. The patient was on 5 mg/day for 6 weeks and then on 10 mg/day for 4

days in study 303 when she patient became nauseated and had a **syncopal spell**. The day prior the patient had nausea and vomiting. No CRF in the CANDA. No additional information found in the paper CRF.

Study 303, patient 152: This was a 72 year old who was on placebo during study 302 and was on treatment in study 303 for almost 15 weeks when he became **agitated** and confused. No CRF in the CANDA. Paper CRF reviewed.

Study 303, patient 224: This was a 77 year old male who was on 10 mg/day in study 301, 5 mg/day for 6 weeks in study 303 and 10 mg/day for 6 weeks when he had an episode of **epigastric pain** with nausea and vomiting. No CRF in the CANDA.

Study 303, patient 300: This was a 72 year old female who was on 5 mg/day in study 301 and titrated to 10 mg/day had a generalized tonic clonic **seizure** about 10 weeks after starting study 303. No CRF in the CANDA. In the paper CRF, the patients was noted to have a syncopal spell. There was no mention of a seizure.

Study 303, patient 313: This is an 81 year old female. It is not noted if this patient was from study 301 or 302. She had been on 10 mg/day for 5 to 6 months when she reported feeling weak and washed out. She had two prior **syncopal episodes**, one that had occurred one day prior and another that occurred 5 months ago. On evaluation her pulse was in the 40s and her BP was 60/palpable. The investigator thought that the events may be related to atenolol. CRF not in the CANDA. No new information in the paper CRF.

Study 303, patient 326-301: This was an 84 year old female who was on 5 mg in study 301 and then on 5 mg for 6 weeks and 10 mg/day for 12 weeks. The patient awoke with a headache and then became unconscious (**syncope**). She had a similar event during study 301. One week later, she had an episode of dizziness and her dose was decreased to 5 mg/day. About 10 weeks later, she complained of chest pain which was diagnosed as angina. Study medication was discontinued at that time.

Study 303, patient 387-301: This was a 90 year old female who was on placebo during study 301, 5 mg/day for 6 weeks and 10 mg/day for 6 weeks in study 303 when she was noted to had a **syncopal episode**. The patient had a fever and was diagnosed with a bacterial meningitis or viral encephalitis. The CRF was not in the CANDA.

Blinded treatment:

Study 304, patient 172: This was an 84 year old male who was receiving blinded

treatment for 9 days when he developed nausea, decreased appetite, dizziness and increased **agitation** followed by increased confusion. The study treatment was stopped for 4 days without improvement in symptoms. 13 days after restarting treatment, the patient was hospitalized for dehydration.

Study 304, patient 253: This was an 80 year old female who had received study treatment for 3 days when the patient **fell** in her bedroom and fractured her femur.

Review And Evaluation Of Clinical Data

NDA:---------- 20-690
Sponsor:---------- Eisai
Drug:---------- E2020
Indication:----------
Review completed:---------- 8/29/96

Introduction:

The following additional information is provided to supplement my review.

Exposure to the drug in the Japanese studies:

The dose and duration for the phase 1 studies and the phase 2 Japanese studies are provided in the following tables.

Dose and duration of exposure in phase 1 studies (some subjects may have received more than one dose)

	Plb	0.1 to 1 mg	2 to 4 mg	5 mg	6 to 9 mg	10 mg	Total
One day	8	30	36	144	6	6	230
21 days	9	6	6	6			27
28 days				14		14	28
Total	17	36	42	164	6	20	285

Dose and Duration for the Japanese phase 2 studies

	Plb	≤ 1 mg	2 mg	3 mg	5 mg	Total
8 weeks		31		9		40
12 weeks		33	31	2	18	84
16 weeks	60			66	64	190
38 weeks	19		19			38
48 weeks			46[a]			46
Total	79	64	96	77	82	398

[a]38 patients treated in study 131A were subsequently enrolled into study 131B.

The dose and duration of exposure in the phase 2/3 studies including the Japanese studies is in the following table:

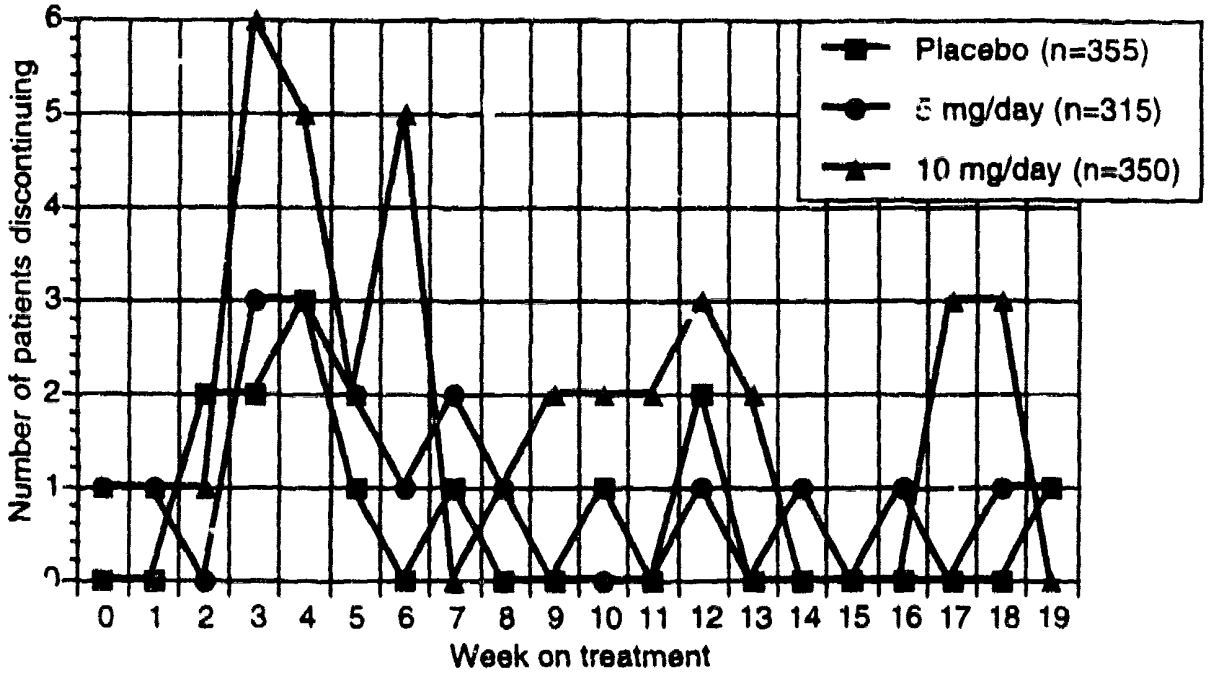
Number of patients exposed to E2020 by dose and duration (studies 201, 202, 301 and 302 and Japanese phase 2 studies)									
	All doses			5 mg			10 mg		
Exposure	Any	≥ 3 months	> 6 months	Any	≥ 3 months	> 6 months	Any	> 3 months	> 6 months
201/202[a]	152	120	103	135	109	96	62	9	0
301	315	256	0	161	136	0	163	120	0
302	311	249	234	154	138	127	157	111	107
Japanese studies[b]	319	279	65	82	82	64	0	0	0
All studies	1097	904	402	532	465	287	382	240	107

[a] in study 202 patients could be on both 5 and 10 mg/day. Patients on 7 mg were included in the 5 mg/day group.
[b]For the Japanese studies, the numbers are based on the planned duration of the study and that all patients enrolled completed the study.

Time course for patients discontinuing:

The number of patients discontinuing treatment in study 201, 301 and 302 at each week of the study is displayed in the following graph.

Study 201, 301 and 302: Number of patients discontinuing at each week of treatment



Baseline characteristics of patients enrolled in the controlled clinical trials:

The baseline characteristics including age, sex, age by sex, height, weight, medical history, baseline severity of Alzheimer's Disease, prior medication usage, and concomitant medication usage were similar between groups. 95% of the patients were white, 80% were between the age of 65 and 85. 60% were female. Approximately 20% of the female patients were on sex replacement hormones. Displays comparing baseline characteristics provided by the sponsor are in Appendix A.

Analysis of safety data by age, sex and race:

In this population, 95% of the patients were white. The small number of patients involved in each these subgroups makes it difficult to assess differences.

In regards to age, there was generally an increase in the incidence of the common AEs (fatigue, anorexia, diarrhea, nausea, insomnia) when patients < 65 were compared to those > 65. The incidence of these AEs and the numbers of patients by age in the controlled studies are summarized in the following table.

Incidence of AEs by age in the controlled studies												
	<65			65-74			75-84			>85		
	Plb	5 mg	10 mg	Plb	5 mg	10 mg	Plb	5 mg	10 mg	Plb	5 mg	10 mg
N	58	50	42	136	130	108	147	151	147	14	19	18
Fatigue	3 (5)	2 (4)	2 (5)	3 (2)	4 (3)	4 (4)	6 (4)	8 (5)	15 (10)	0	0	3 (17)
Anorexia	2 (3)	2 (4)	2 (5)	1 (1)	3 (2)	6 (6)	4 (3)	3 (2)	9 (6)	0	2 (11)	4 (22)
Diarrhea	3 (5)	4 (8)	5 (12)	7 (5)	8 (6)	14 (13)	6 (4)	14 (9)	24 (16)	0	1 (5)	5 (28)
Nausea	5 (9)	3 (6)	5 (12)	5 (4)	8 (6)	22 (20)	8 (5)	8 (5)	29 (20)	2 (14)	2 (11)	4 (22)
Vomiting	3 (5)	3 (6)	2 (5)	1 (1)	4 (3)	6 (6)	5 (3)	3 (2)	17 (12)	2 (14)	0	1 (6)
Muscle Cramp	1 (2)	4 (8)	4 (10)	3 (2)	5 (4)	9 (8)	2 (1)	9 (6)	10 (7)	1 (7)	1 (5)	1 (6)
Insomnia	3 (5)	1 (2)	3 (7)	8 (6)	10 (8)	13 (12)	9 (6)	11(7)	21 (14)	0	0	7 (39)

Approximately 60% of the patients enrolled in the controlled clinical trials were female. In general, there was an increase in the common AEs (fatigue, anorexia, nausea, vomiting and weight decrease) in females compared to males. The results are summarized in the following table:

Incidence of AEs by sex in the controlled studies						
	Males			Females		
	Plb	5 mg	10 mg	Plb	5 mg	10 mg
Total Number of Patients	142	120	122	213	230	193
Number (%) with at Least One AE	96 (68)	81 (68)	86 (70)	158 (74)	169 (73)	165 (85)
Fatigue	3 (2)	2 (2)	5 (4)	9 (4)	12 (5)	19 (10)
Headache	7 (5)	9 (8)	12 (10)	25 (12)	21 (9)	25 (13)
Anorexia	3 (2)	4 (3)	4 (3)	4 (2)	6 (3)	17 (9)
Diarrhea	6 (4)	10 (8)	18 (15)	10 (5)	17 (7)	30 (16)
Nausea	7 (5)	2 (2)	12 (10)	13 (6)	19 (8)	48 (25)
Vomiting	3 (2)	1 (1)	3 (2)	8 (4)	9 (4)	23 (12)
Weight Decrease	2 (1)	1 (1)**	4 (3)	3 (1)	4 (2)**	11 (6)
Muscle Cramp	4 (3)	7 (6)	9 (7)	3 (1)	12 (5)	15 (8)
Insomnia	10 (7)	10 (8)	14 (11)	10 (5)	12 (5)	30 (16)

Analysis of efficacy data by age, sex and other baseline characteristics:

The sponsor performed analyses evaluating the efficacy results based on age, sex and baseline disease severity scores (CDR and MMSE). When these values were used as covariates in the ANCOVA model, the differences between groups remained statistically significant suggesting that these variables did not explain the treatment effect.

The ADAS-COG analyses in studies 301 and 302 of subgroups based on sex, baseline CDR score and age group demonstrated treatment differences in favor of active treatment in the various subgroups. Except in the youngest and oldest age groups where the number of patients were relatively small, the differences were associated with p values < 0.05 except in study 301, the treatment effect seen with patients in the 5 mg group with baseline CDR score of 2 did not reach a p value of < 0.05.

The CIBIC plus analyses in studies 301 and 302 of subgroups based on sex, baseline CDR and age demonstrated treatment differences in favor of active treatment in the subgroups. The effects generally were not associated with a nominal p value of < 0.05 except for the largest groups such as female patients and patients in their 70s and patients with a CDR of 1 at screening.

Results of QOL:

The QOL was a 7 or 8 items (Studies 301 and 302 used the 7 item scale and Study 201, the 8 item scale) measure that was intended to capture the patient's perception of the following areas: relationships, eating and sleeping and social/leisure activity. This test was conducted through an interview with the patient by a nurse/evaluator or clinician. In study 201, a separate interview was conducted with the caregiver to determine their perception of the patient's QOL.

QOL measurements in study 301 showed an overall improvement in patients on placebo and 5 mg/day and worsening in patients on 10 mg/day. The differences between placebo. Though the treatment differences were associated with p values > 0.05, in the LOCF population, the comparison between placebo and 10 mg/day was associated with a p value < 0.05. In study 302, patients on 5 had improvement while patients on 10 mg/day and placebo worsened. The 10 mg group score was worse than placebo. These differences were associated with a nominal p value of $> .05$. In study 201, patients on drug showed improvement

while patients on placebo showed worsening. For the QOL measure that included input from the caregiver, both the placebo and 5 mg group had improvement though the placebo group had an overall better score. This difference was associated with a p value > 0.05.

Overall, in the QOL measure with mean baseline scores of about 250 to 300 (SE about 2 to 4 points), mean differences between groups ranged from about 1 to 10 points. In study 301 the difference between the 10 mg group and placebo was about 8 points in favor of placebo. In study 302, the difference was about 2 points in favor of placebo. In study 201, the difference was about 4 points in the caregivers assisted rating in favor of placebo and about 10 in the patient's rated assessment in favor of the drug.

Because of the cognitive problems in this population, patients are not reliable sources of information on QOL and caregivers may have a difficult time assessing a patient's QOL. This may explain the high variability of scores and the inconsistency of these results with the primary outcome measures.

Randy Levin, M.D.
Medical Reviewer

cc:
NDA 20-690
HFD-120
rl/August 29, 1996

Appendix A:

Baseline characteristics of patients in the controlled clinical trials

Table 5A

Summary of Demographic Characteristics Controlled Studies

Characteristics	Number (%) of Patients						
	Total	Placebo	E2020 1 mg/day	E2020 3 mg/day	E2020 5 mg/day	E2020 10 mg/day	All E2020 Groups
N	1102	355	42	40	350	315	747
Age (Years)							
Mean	73	73	73	71	73	74	73
Min., Max.	50-94	52-93	55-85	54-85	50-94	50-94	50-94
<65	167 (15)	58 (16)	8 (19)	9 (23)	50 (14)	42 (13)	109 (15)
65-74	406 (37)	136 (38)	16 (38)	16 (40)	130 (37)	108 (34)	270 (36)
75-84	474 (43)	147 (41)	15 (36)	13 (33)	151 (43)	147 (47)	327 (44)
≥85	55 (5)	14 (4)	2 (5)	2 (5)	19 (5)	18 (6)	41 (5)
Sex							
Male	415 (38)	142 (40)	13 (31)	18 (45)	120 (34)	122 (39)	273 (37)
Female	687 (62)	213 (60)	29 (69)	22 (55)	230 (66)	193 (61)	474 (63)
Race							
White	1052 (95)	340 (96)	41 (98)	37 (93)	332 (95)	302 (96)	712 (95)
Black	32 (3)	12 (3)	1 (2)	3 (8)	12 (3)	4 (1)	20 (3)
Other	18 (2)	3 (1)	0	0	6 (2)	9 (3)	15 (2)

Data Source: Table 3.0.

Table 5C

Severity of Disease at Screening
Controlled Studies

	Number (%) of Patients						
Measure	Total	Placebo	E2020 1 mg/day	E2020 3 mg/day	E2020 5 mg/day	E2020 10 mg/day	All E2020 Groups
CDR							
N	1102	355	42	40	350	315	747
0.5	9(1)	2(1)	1(2)	1(3)	2(1)	3(1)	7(1)
1	838(76)	274(77)	32(76)	29(73)	264(75)	239(76)	564(76)
2	254(23)	79(22)	9(21)	10(25)	84(24)	72(23)	175(23)
MMSE							
N	1100	355	42	40	350	313	745
Mean	19	19	19	18	19	19	19
Min. Max.	8-27	9-26	10-26	10-26	9-26	8-27	8-27

Note: CDR is the Clinical Dementia Rating; MMSE is the Mini-Mental Status Examination.
Data Source: Table 3.0.

Table 5D

Abnormal Medical Histories Reported by at Least 30% of any Treatment Group at Screening
Controlled Studies

Medical History	Number (%) of Patients					
	Placebo	E2020 1 mg/day	E2020 3 mg/day	E2020 5 mg/day	E2020 10 mg/day	All E2020 Groups
Total	355	42	40	350	314[1]	746[1]
Number (%) of Patients with any Abnormal History (Excluding Neurological)	351 (99)	42 (100)	37 (93)	341 (97)	312 (99)	732 (98)
Musculoskeletal	190 (54)	24 (57)	18 (45)	200 (57)	182 (58)	424 (57)
Cardiovascular	166 (47)	16 (38)	17 (43)	187 (53)	150 (48)	370 (50)
Ophthalmologic	175 (49)	18 (43)	15 (38)	174 (50)	161 (51)	368 (49)
Gastrointestinal	164 (46)	23 (55)	15 (38)	156 (45)	156 (50)	350 (47)
Other	169 (48)	20 (48)	19 (48)	164 (47)	145 (46)	348 (47)
Renal/Genitourinary	178 (50)	18 (43)	18 (45)	159 (45)	146 (46)	341 (46)
Allergy	108 (30)	8 (19)	5 (13)	104 (30)	84 (27)	201 (27)

[1]Medical history not completed for one patient from the 10 mg/day group.
Data Source: Table 5.0.

Table 5E

Prior Medications Used by at Least 2% of all E2020 Groups Combined Controlled Studies

Therapeutic Class	Number (%) of Patients					
	Placebo	E2020 1 mg/day	E2020 3 mg/day	E2020 5 mg/day	E2020 10 mg/day	All E2020 Groups
Total	355	42	40	350	315	747
Total Who Took Medications	62 (17)	10 (24)	11 (28)	73 (21)	69 (20)	157 (21)
Analgesics	8 (2)	1 (2)	0	11 (3)	9 (3)	21 (3)
Antibacterials for Systemic Use	13 (4)	2 (5)	1 (3)	9 (3)	9 (3)	21 (3)
Neuroleptics[1]	8 (2)	1 (2)	1 (3)	10 (3)	9 (3)	21 (3)
Antihistamines for Systemic Use	4 (1)	0	1 (3)	9 (3)	7 (2)	17 (2)
Antiinflammatory and Antirheumatic Products	1 (< 1)	0	0	10 (3)	3 (1)	13 (2)
Psychoanaleptics	7 (2)	2 (5)	1 (3)	6 (2)	3 (1)	12 (2)

[1]ATC term is Psycholeptics.
Note: Prior medications are those that were discontinued sometime within the month preceding the Screening visit.
Data Source: Table 6.0.

Table 5F

Concomitant Medications Used by at Least 5% of any Treatment Group Controlled Studies

Therapeutic Class	Number (%) of Patients					
	Placebo	E2020 1 mg/day	E2020 3 mg/day	E2020 5 mg/day	E2020 10 mg/day	All E2020 Groups
Total	355	42	40	350	315	747
Total Who Took Medications	316(89)	34(81)	33(83)	303(87)	279(87)	643(86)
Analgesics	158(45)	14(33)	13(33)	139(40)	139(44)	305(41)
Vitamins	116(33)	12(29)	7(18)	98(28)	101(32)	218(29)
Antiinflammatory and Antirheumatic Products	52(15)	10(24)	2(5)	56(16)	64(20)	132(18)
Antibacterials for Systemic Use	68(19)	5(12)	8(20)	58(17)	59(19)	130(17)
Calcium Channel Blockers	44(12)	3(7)	1(3)	47(13)	39(12)	90(12)
Antacids, Drugs for Treatment of Peptic Ulcer and Flatulence	50(14)	4(10)	4(10)	41(12)	40(13)	89(12)
Mineral Supplements	30(8)	6(14)	0	38(11)	38(12)	82(11)
Diuretics	46(13)	2(5)	4(10)	39(11)	32(10)	77(10)
Neuroleptics[1]	36(10)	4(10)	4(10)	30(9)	37(12)	75(10)
Sex Hormones and Modulators of the Genital System	33(9)	6(14)	4(10)	29(8)	36(11)	75(10)
Thyroid Therapy	38(11)	3(7)	1(3)	30(9)	35(11)	69(9)
Antihypertensives	28(8)	2(5)	2(5)	37(11)	21(7)	62(8)
Cardiac Therapy	30(8)	1(2)	1(3)	32(9)	23(7)	57(8)
Laxatives	25(7)	0	6(15)	26(7)	25(8)	57(8)
Ophthalmologicals	36(10)	0	0	34(10)	21(7)	55(7)
Cough and Cold Preparations	33(9)	1(2)	5(13)	23(7)	24(8)	53(7)

[1]ATC term is Psycholeptics.
Data Source: Table 7.0

Table 5F (Cont'd)

Concomitant Medications Used by at Least 5% of any Treatment Group Controlled Studies

Therapeutic Class	Number (%) of Patients					
	Placebo	E2020 1 mg/day	E2020 3 mg/day	E2020 5 mg/day	E2020 10 mg/day	All E2020 Groups
Serum Lipid Reducing Agents	19(5)	3(7)	1(3)	21(6)	21(7)	46(6)
Antihistamines for Systemic Use	12(3)	2(5)	3(9)	13(4)	19(6)	37(5)
Antianemic Preparations	21(6)	1(2)	1(3)	13(4)	19(6)	34(5)
Antidiarrheal, Intestinal Anti-inflammatory/ Antiinfective Agents	11(3)	0	0	14(4)	19(6)	33(4)
Beta Blocking Agents	22(6)	1(2)	2(5)	12(3)	12(4)	27(4)
Vaccines	16(5)	0	0	14(4)	12(4)	26(3)
Nasal Preparations	7(2)	0	2(5)	10(3)	6(2)	18(2)

[1]ATC term is Psycholeptics.
Data Source: Talk 7.0

Randy Levin, M.D.

Diggins

RETURN

JUN 20 1996

Statistical Review and Evaluation

JUN 20 1996

NDA#: 20-690

Applicant: Eisai America, Inc.

Name of Drug: Aricept

Documents Reviewed: Vols 1.105, 1.127

Medical Officer: Randy Levin, M.D., HFD-120

Background

The sponsor has submitted two randomized, placebo controlled, multicenter trials (301 & 302) in support of Aricept as a treatment for the symptoms of Alzheimer's Disease. The statistical results clearly demonstrate a difference of both the 5mg/day and 10mg/day doses from placebo. There are no substantial statistical issues. Consequently, this review briefly reports the primary methodology and results of the trials. All tables and graphics are taken from the sponsor's study reports.

Results of the Trials

Trial 301 had a 12-week double blind period and a subsequent 3-week washout period. Trial 302 had a 24-week double blind period and a 6-week washout period. Tables 1a and 1b display the numbers of patients in trials 301 and 302, respectively. In addition, there were no substantial differences among treatment arms in severity of illness. Tables 2a and 2b display the results for change from baseline in the sum of ADAS-COG scores. Analyses were done using ANCOVA with baseline ADAS-COG as the covariate. Results were very similar regardless of whether the observed cases or the LOCF data were analyzed. Although numerically superior, 10mg/day was not statistically superior to 5mg/day in both trials. Figures 1a and 1b display the ADAS-COG results over time. In Figure 1b, the active treatment groups regress to the placebo group after 6-weeks off-drug (Trial 302).

Tables 3a and 3b display the results of the CIBIC+ (Clinicians 'global assessment') for observed cases and LOCF. Figures 2a and 2b display the histograms for the CIBIC+ scores. Note a general shift to the left for both active groups relative to placebo.

Conclusion

Both 5mg/day and 10mg/day Aricept demonstrate statistically significant differences from placebo on the two primary clinical endpoints in both trials. There was no evidence of treatment by gender interaction.

Table 1a
(301)

Summary of Demographic Characteristics
Number (%)

Characteristic	Treatment			Total
	Placebo	E2020 5 mg/day	E2020 10 mg/day	
Number of patients	153	157	158	468
Age (Years)				
Mean	73.98	73.82	73.39	73.73
Range	52-93	50-94	50-92	50-94
<65	22 (14)	18 (11)	22 (14)	62 (13)
65-74	55 (36)	64 (41)	55 (35)	174 (37)
75-84	66 (43)	62 (39)	72 (46)	200 (43)
≥85	10 (7)	13 (8)	9 (6)	32 (7)
Sex				
Male	60 (39)	49 (31)	62 (39)	171 (37)
Female	93 (61)	108 (69)	96 (61)	297 (63)
Race				
White	147 (96)	149 (95)	152 (96)	448 (96)
Black	6 (4)	6 (4)	1 (1)	13 (3)
Other	0	2 (1)	5 (3)	7 (1)

Table 1b
(302)

Demographic Characteristics by Treatment Group
Number (%)

Characteristic	Placebo	E2020 5 mg/day	E2020 10 mg/day	All Treatments
Number of Patients Enrolled	162	154	157	473
Mean Age	72.62	72.85	74.58	73.35
Sex				
Male	63 (39)	57 (37)	60 (38)	180 (38)
Female	99 (61)	97 (63)	97 (62)	293 (62)
Race				
White	153 (94)	146 (95)	150 (96)	449 (95)
Black	6 (4)	5 (3)	3 (2)	14 (3)
Other	3 (2)	3 (2)	4 (3)	10 (2)

Summary of ADAS-cog Assessments - ITT Population

Visit	Placebo n	Placebo Mean (SE)	E2020 5mg/day n	E2020 5mg/day Mean (SE)	E2020 10mg/day n	E2020 10mg/day Mean (SE)	Overall Treatment Effect
Baseline	150	25.28	156	26.39	155	26.41	p=0.4916
S.E. of Mean		(0.87)		(0.92)		(0.89)	
Mean Change from Baseline at Week 12	139	0.46	141	-2.23	125	-2.71	
LS Mean Change		0.40		-2.23		-2.77	p<0.0001
S.E. of LS Mean		(0.46)		(0.46)		(0.49)	
p-value vs. Placebo			p<0.0001		p<0.0001		
Mean Change from Baseline at Endpoint	150	0.39	154	-2.09	153	-2.73	
LS Mean Change		0.36		-2.08		-2.71	p<0.0001
S.E. of LS Mean		(0.43)		(0.43)		(0.43)	
p-value vs. Placebo			p<0.0001		p<0.0001		

p-values based on an ANACOVA using Fisher's two-tailed least significant difference procedure for the pairwise comparisons

Table 2b
(302)

Summary of ADAS-cog Assessments - ITT Population

Visit	Placebo n	Placebo Value	E2020 5 mg/day n	E2020 5 mg/day Value	E2020 10 mg/day n	E2020 10 mg/day Value	Overall Treatment Effect [a]
Baseline	154	27.28	153	26.28	151	27.41	p=0.6850
S.E. of Mean		(0.96)		(0.96)		(0.86)	
Mean Change from Baseline at 24 Week	132	1.92	130	-0.87	105	-1.32	
LS Mean Change		1.81		-0.90		-1.34	p<0.0001
S.E. of LS Mean		(0.51)		(0.51)		(0.57)	
p-value vs. placebo [a]			p=0.0001		p<0.0001		
Mean Change from Baseline at Endpoint	153	1.73	152	-0.79	150	-1.14	
LS Mean Change		1.82		-0.67		-1.06	p<0.0001
S.E. of LS Mean		(0.49)		(0.51)		(0.51)	
p-value vs. placebo [a]			p<0.0001		p<0.0001		

Data Source: Table 4.0

[a] p-values based on an ANACOVA using Fisher's two-tailed least significant difference procedure for the pairwise comparisons

Table 3a
(301)

Summary of CIBI-C Plus Assessments - ITT Population

Visit	Placebo n	Placebo Mean (SE)	E2020 5mg/day n	E2020 5mg/day Mean (SE)	E2020 10mg/day n	E2020 10mg/day Mean (SE)	Overall Treatment Effect
Mean at Week 12	137	4.19	141	3.85	126	3.81	p=0.0025
S.E.		(0.08)		(0.08)		(0.08)	
p-value vs. Placebo				p=0.0010		p=0.0088	
Mean at Endpoint	150	4.19	153	3.90	152	3.85	p=0.0058
S.E.		(0.07)		(0.08)		(0.08)	
p-value vs. Placebo				p=0.0026		p=0.0082	

CIBI-C Plus: 0=Not assessed; 1=Marked Improvement; 2=Moderate Improvement; 3=Minimal Improvement; 4=No Change; 5=Minimal Worsening; 6=Moderate Worsening; 7=Marked Worsening
p-value from a Cochran-Mantel Haenszel test excluding not assessed.

Table 3b
(302)

Summary of CIBI-C Plus Assessments - ITT Population

Visit	Placebo n	Placebo Mean (SE)	E2020 5 mg/day n	E2020 5 mg/day Mean (SE)	E2020 10 mg/day n	E2020 10 mg/day Mean (SE)	Overall Treatment Effect [a]
Mean at Week 24	133	4.53	131	4.18	103	4.10	p=0.0011
S.E.		(0.09)		(0.09)		(0.09)	
p-value vs. placebo [a]				p=0.0063		p=0.0003	
Mean at Endpoint	152	4.51	149	4.15	149	4.07	p<0.0001
S.E.		(0.08)		(0.09)		(0.07)	
p-value vs. placebo [a]				p=0.0047		p<0.0001	

Data Source: Table 5.0
Note: CIBI-C Plus: 0=Not Assessed; 1=Marked Improvement; 2=Moderate Improvement; 3=Minimal Improvement; 4=No Change; 5=Minimal Worsening; 6=Moderate Worsening; 7=Marked Worsening
[a] p-values from a Cochran-Mantel-Haenszel test excluding not assessed

Figure 1a
(301)

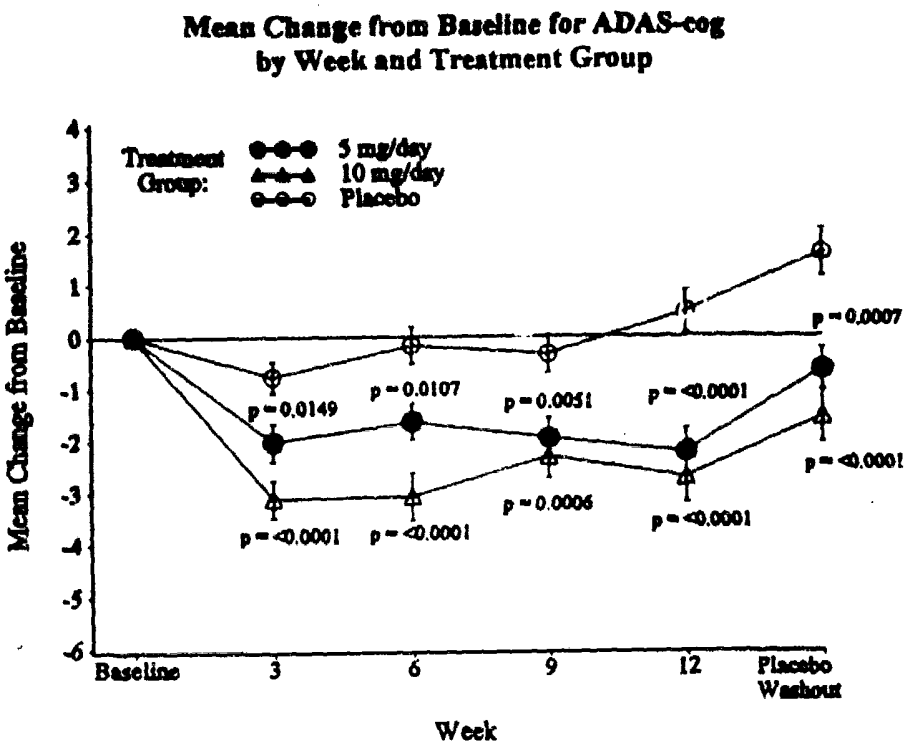


Figure 1b
(302)

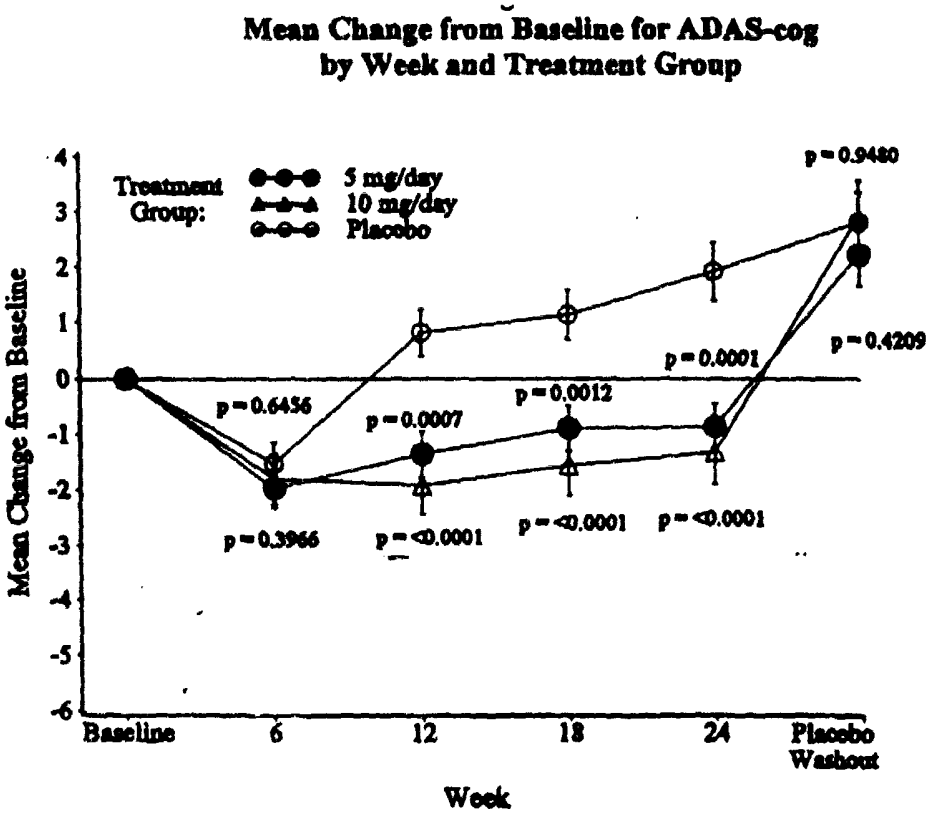


Figure 2a
(301)

Clinician's Interview-Based Impression of Change by Treatment Group at Endpoint

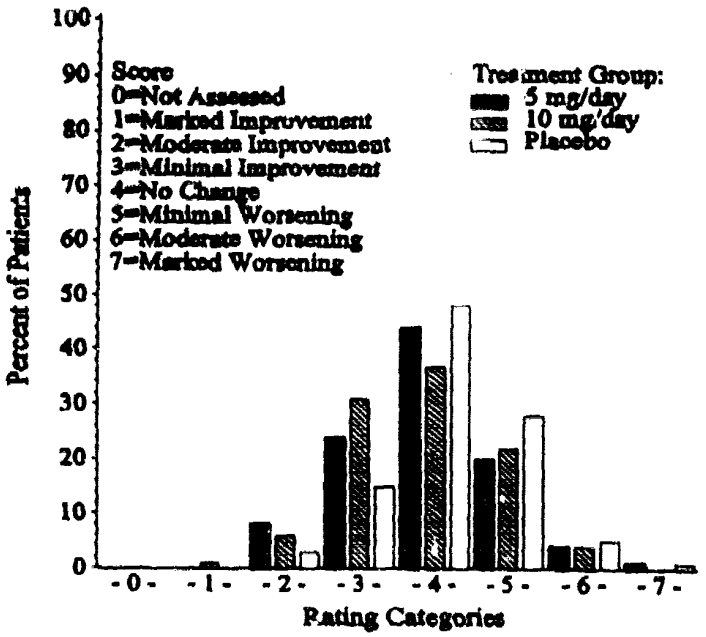
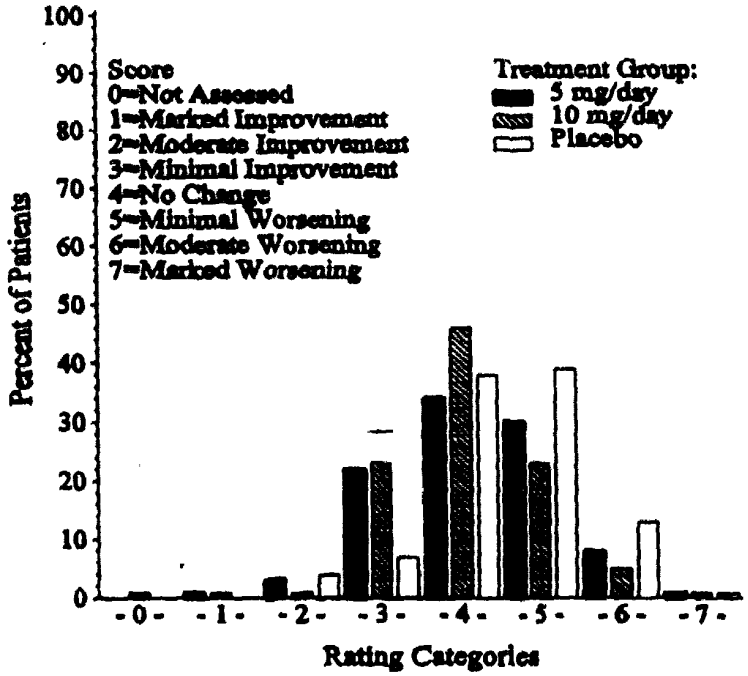


Figure 2b
(302)

Clinician's Interview-Based Impression of Change by Treatment Group at Endpoint



BIO Review

NOV 8 1996

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA 20,690
Donepezil HCl (Aricept®)
5, 10 mg Tablets

Eisai America Inc., DCTIIDN HT
300 Frank W. Burr Blvd.
Teaneck, NJ 07666 NOV 13 1996

Reviewer: Vijay K. Tammara, Ph. D.

Submission Date:
October 03, 1996

Indication: Alzheimer's

Type of Submission: Responses to FDA's Approvable Letter

In this submission, the sponsor has provided responses to FDA's Approvable Letter dated September 19, 1996. The sponsor has agreed to adopt the dissolution methodology and specification as outlined in the Biopharmaceutics section of the Approvable Letter (Attachment 1). The sponsor also responded satisfactorily to other comments as outlined in FDA's Approvable Letter and committed to perform appropriate studies (Attachment 1).

Recommendation:

The sponsor has responded satisfactorily to all the Biopharmaceutics comments.

11/08/96

Vijay K. Tammara, Ph. D.
Division of Pharmaceutical Evaluation I

RD/FT initialed by M. Hossain, Ph. D. 11/8/96

CC: NDA 20-690 (orig.), HFD-860 (Tammara, Hossain, Malinowski), HFD-870 (Drug, Chron, and Reviewer files).

COMPLETED AUG 1 1996

AUG 0 1 1996

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

Donepezil Hydrochloride (E2020; Aricept®)
5 and 10 mg Tablets
NDA 20-690
6741

Eisai America Inc.,
300 Frank W. Burr Blvd.
Teaneck, NJ 07666-

Reviewer: Vijay K. Tammara, Ph.D.
Indication: Alzheimer's Dementia
Classification: 1P
Type of Submission: Original--New Molecular Entity

Submission Dates:
March 29, 1996
May 01, 1996
May 02, 1996
May 13, 1996
June 03, 1996
June 04, 1996
July 02, 1996
July 03, 1996
July 09, 1996

SYNOPSIS

Donepezil HCl (commonly referred to as E2020) is a piperidine-based reversible inhibitor of the enzyme acetylcholinesterase (AChE). The mechanism of action is presumably due to dose-dependent inhibition of AChE resulting in enhanced cholinergic function in the central nervous system and thereby exerting its therapeutic effect. The starting dose is 5 mg daily (one 5 mg tablet) to avoid adverse cholinergic effects, which then may be increased to 10 mg after at least one month administration of 5 mg/day. E2020 is a racemate consisting of two enantiomers, the R and S enantiomers, and both enantiomers inhibit AChE to the same extent. The focus of this NDA is racemate E2020.

The sponsor had conducted clinical/pharmacokinetic studies using the film coated tablets manufactured at Kawashima, Japan. The eventual to-be-marketed dosage form will be manufactured in US at either or Pfizer. In this regard, the sponsor has performed single dose bioequivalence studies involving these two sites of manufacture vs Kawashima. The film-coated tablets manufactured at different sites were bioequivalent based on log transformed $AUC_{0\text{-}168hr}$ and Cmax, and also untransformed Tmax and $AUC_{0-\infty}$.

E2020 is well absorbed with a relative oral bioavailability of about 101% compared to an oral solution and reaches peak plasma concentrations in 3 to 4 hours. Neither food nor time of administration (morning vs evening dose) has

influence on the pharmacokinetics of E2020. The variability in the pharmacokinetic parameters across the studies was found to be consistent and the coefficient of variation was in the range of 10-25%.

E2020 distributes extensively into tissues as evidenced by a large apparent volume of distribution of 12 L/Kg. E2020 binds to plasma proteins extensively (96%), mainly to albumins (about 75%) and α_1-acid glycoprotein (about 21%) over the concentration range of 2 -1000 ng/mL.

E2020 is both excreted in the urine intact and extensively metabolized to four major metabolites and a number of minor metabolites, not all of which have been identified. The metabolism of E2020 consists of CYP 450 dependent oxidation and glucuronidation. Following a single 5 mg oral dose of ^{14}C-E2020, an average of 72% of total radioactivity was recovered in urine (57%) and feces (15%) over 10 days, while 28% remained unrecovered. Unchanged drug is the primary component in plasma and was calculated to be about 53% of the total plasma radioactivity. The metabolites (identified and unidentified) accounted for the remainder 47% of total radioactivity in plasma. Of all the metabolites, only M1 (which accounted for at least 11% of total radioactivity) has been reported to inhibit AChE to the same extent as E2020 *in vitro* and was found to be present in plasma at concentrations equal to about 20% of the concentrations observed of E2020. The mean elimination half-life of E2020 is about 70 hours (CV = 18%).

The kinetics of E2020 was found to be proportional to dose at steady state over the dose range of 1-10 mg when given once daily. Steady state was observed to be reached within 15 days. Following administration of either 5 or 10 mg doses once daily, an accumulation ratio of about 4 for Cmax and about 7 for AUC was observed. Further, steady-state CSF trough concentrations appear to be proportional to dose and average CSF: Plasma E2020 ratio was 16%. Hepatic and renal impairment; age, gender, and race does not appear to affect the pharmacokinetics of E2020.

The pharmacokinetic and pharmacodynamic relationship established by the Emax model indicated a significant relationship between E2020 plasma concentrations and the percent inhibition of AChE and demonstrated no time dependent changes in E2020 pharmacodynamics. Mean steady state plasma concentrations o' the 5 mg/day and 10 mg/day doses, resulted in a mean steady state AChE inhibition respectively. Inhibition of AChE is comparable between healthy subjects and patients, and remains stable over time.

In vivo drug interaction studies indicated that cimetidine and digoxin did not significantly affect the pharmacokinetics of E2020; similarly E2020 did not affect the pharmacokinetics of cimetidine, digoxin, theophylline and warfarin. *In vitro* drug displacement studies indicated that E2020 at concentrations of did not affect the binding of furosemide , digoxin ng/mL), and warfarin , to human albumin and vice versa.

E2020 was quantified in plasma, whole blood, urine, and feces by and the limit of quantitation Overall, the analytical validation was found to be satisfactory in terms of specificity, sensitivity, linearity, precision, and accuracy. An equilibrium dialysis method was used to support *in vitro* protein binding determinations.

RECOMMENDATION:

This submission (NDA 20-690) has been reviewed by the Office of Clinical Pharmacology and Biopharmaceutics' and has been found to be acceptable for meeting the Offices' requirements, provided the sponsor incorporates all the labeling changes and responds satisfactorily to all enclosed Comments. The biowaiver request for the 10 mg tablet could not be granted at this time due to lack of multi-media dissolution profiles. The sponsor is requested to adopt the "interim" dissolution methodology and specification as outlined in Comment 9. Please forward Comments 1-9 and this Recommendation to the sponsor.

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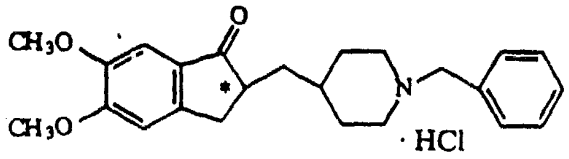
(The above Appendices are available in the Office of Clinical Pharmacology and Biopharmaceutics archive files. A total of 25 studies were submitted, of which 4 were found to be repetitive and pilot in nature, and hence only 21 studies were reviewed).

INTRODUCTION:

Donepezil hydrochloride (commonly referred to as E2020) is a piperidine-based reversible inhibitor of the enzyme acetylcholinesterase (AChE), known chemically as (±)2,3-dihydro-5,6-dimethoxy-2[[1-phenylmethyl)-4-piperidinyl]methyl]-1H-inden-1-one Hydrochloride. Its molecular formula is $C_{24}H_{29}NO_3HCl$ and the molecular weight is 415.96.

E2020 is a white crystalline powder and is freely soluble in chloroform, highly soluble (16 mg/mL) in water, 0.1 N HCL, and in glacial acetic acid, slightly soluble in ethanol and in acetonitrile and practically insoluble in ethyl acetate and in n-hexane. The pKa of E2020 is 8.9 with a log P of 4.27 (octanol-water partition coefficient). Its structural formula is as follows:



DOSAGE FORMS AND ADMINISTRATION

Most of the pharmacokinetic studies were carried out after oral administration of E2020 film coated tablets. Tablets manufactured at different sites were used in the clinical studies.

In the study involving ^{14}C-E2020 administration, the active ingredient was administered in solution form. Lastly, in the bioequivalence studies, the final 5 mg to be marketed dosage form and Pfizer, USA) was compared to 5 mg tablets (Kawashima, Japan) used in the pivotal Phase 3 studies.

MANUFACTURERS: Manufactured by and Pfizer Inc., Brooklyn, NY, USA.

SUMMARY OF HUMAN BIOAVAILABILITY, PHARMACOKINETICS, AND PHARMACODYNAMICS

I. BIOAVAILABILITY:

A. Relative Bioavailability:

In an open, randomized, two-period, single dose, cross over study involving 15 healthy male and female volunteers, the mean relative bioavailability following oral administration of 5 mg film coated tablets relative to an oral solution was 101% (CV = 12%) (Study E2020-A001-008).

B: Bioequivalence:

In the bioequivalence study (E2020-A001-009) , the 5 mg tablets (formulation A; Eisai at Kawashima, Japan) used in the pivotal clinical trials (E2020-A001-301 and 302) was compared with the 5 mg tablets (formulations B and C) produced by , and by respectively. For the purposes of this NDA, the data for formulations A and B is only considered. This study was conducted as a single-dose randomized, open-label, three-period cross over study in 18 healthy male subjects. Using formulation A as the reference treatment for statistical comparisons, formulation B was found to be bioequivalent in terms of log transformed extent of absorption, i.e., $AUC_{0\text{-}168hr}$ (90% C.I. = 99-108%); C_{max} (90% C.I. = 97-106%); and untransformed $AUC_{0-\infty}$ (90% C.I. = 99-109%) and Tmax (90% C.I. = 88-112%).

In another bioequivalence study (E2020-A001-010), the 5 mg tablets (formulation A) used in the pivotal clinical trials (E2020-A001-301 and 302) and produced by Eisai at Kawashima, Japan was compared with the 5 mg tablets (formulation D) produced by Pfizer, Inc., Brooklyn, NY, USA. This study was conducted as a randomized, single-dose, open-label, two-period cross over study in 12 healthy male subjects. Using formulation A as the reference treatment for statistical comparisons, formulation D was found to be bioequivalent in terms of log transformed extent of absorption, i.e., $AUC_{0\text{-}168hr}$ (90% C.I. = 94-100%); C_{max} (90% C.I. = 94-102%);and untransformed $AUC_{0-\infty}$ (90% C.I. = 99-104%) and Tmax (90% C.I. = 88-112%).

C: Waiver Request: The sponsor intend to market a new dosage strength of 10 mg and did not perform any bioequivalence study for this dosage strength. The 10 mg tablet is compositionally proportional to the 5 mg tablet; E2020 exhibits

linear pharmacokinetics over the proposed dosage regimen; and the drug appears to be highly soluble and highly permeable. In order to grant a Biowaiver, the sponsor was requested to submit multi-media dissolution profiles for the 10 mg tablet.

D: Interaction with Food:

In a randomized, open-label, single oral dose, two period cross over study in which a 2 mg tablet was administered to 12 healthy Japanese male subjects, a Japanese breakfast (consisting of 2 pieces of roll, jam, scrambled eggs, sauteed ham, lettuce, cucumber, tomato, and beef consomme soup) was shown to have no affect on the pharmacokinetics of E2020 (E2020-J081-002).

II. PHARMACOKINETICS:

A. Absorption:

Radiolabeled E2020 is well absorbed with less than 1% of the radioactive oral dose recovered in the feces as unchanged drug over a period of 10 days (Study E2020-A001-004). Following oral administration, average peak plasma concentration occurs in about 3 to 4 hours (Studies E2020-A001-002, 008, and 011).

B. Distribution:

In Vivo

E2020 is characterized by a large volume of distribution. The extent and degree of E2020's distribution within various body compartments has not been systematically studied in humans. Following once daily oral administration of 5 mg and 10 mg tablets over 28 days (n = 12 healthy male subjects; Study E2020-A001-011), mean apparent steady-state volume of distribution (Vz/F) was found to be 12 L/Kg (CV = 17%). Further, the mean protein binding using steady state plasma concentrations was calculated to be about 96%.

In Vitro Protein Binding:

In human serum, about 96% of E2020 is bound to plasma proteins mainly to albumin (about 71%) and α_1-acid glycoprotein (about 25%) over the *in vitro* concentration range of 2-1000 ng/mL and binding does not appear to be saturable (Study 19952677).

C. Metabolism :

In Vivo

E2020 is highly metabolized mainly to 4 major metabolites: 6-O-desmethyl donepezil (M1), 5-O-desmethyl donepezil (M2), N-debenzyl donepezil (M4) and donepezil-cis-N-oxide (M6) and a number of minor metabolites (Study E2020-A001-004). Of these metabolites, only M1 has been reported to inhibit AChE to the same extent as E2020 *in vitro* and was found to be present in plasma at concentrations equal to about 20% of E2020. The metabolism of E2020 is mostly hepatic and consists of cytochrome P450-dependent desmethylation via CYP 2D6 (metabolites M1 and M2) and N-debenzylation (metabolite M4) or N-oxidation (metabolite M6) via CYP3A4. Free hydroxyl groups of metabolites M1 and M2 undergo conjugation with glucuronic acid yielding conjugates M11 and M12, respectively. Following a single 5 mg oral dose of ^{14}C-E2020, an average of 72% of total radioactivity was recovered in urine (57%) and feces (15%) over 10 days, while 28% remained unrecovered. About 17% of E2020 dose is recovered in urine as unchanged drug. Comparison of E2020 pharmacokinetics based on specific assay for E2020 plasma concentrations and total plasma radioactivity indicated that unchanged drug is the primary component in plasma and was calculated to be about 53%(CV = 18%). The metabolites (identified and unidentified) accounted for the remainder 47% of total radioactivity in plasma, of which the major component was metabolite M1 and was accounted for at least 11% of total radioactivity in plasma.

In Vitro

In vitro experiments using human liver microsomes showed that desmethylation (metabolites M1 and M2) and N-debenzylation (M4) or N-oxidation (M6) were the major metabolic pathways in human (Study 19952792). Further, the results of this study showed that CYP 2D6 was the principal enzyme which mediated the desmethylation of E2020 (10 μM) resulting in the two major desmethyl metabolites M1 and M2, while CYP3A4 was the principal enzyme which mediated the formation of the other two metabolites, M4 and M6. However, the concentrations of Aricept used were so high (10 μM) that switchability from a high affinity, low capacity enzyme (e.g., CYP 2D6) to a low affinity, high capacity enzyme (e.g., CYP 3A4) is a clear possibility. Therefore, conducting *in vitro* studies using relevant therapeutic concentrations of Aricept ($\leq$164nM) may be useful to determine the specific isozyme responsible for the metabolism of Aricept.

In an *in vitro* study (Study D23) using hepatic microsomes, the possibility of metabolic drug interactions of E2020 with human cytochrome P450s (1A2,

2C9, 2C19, 2D6, and 3A4) was determined to be low. The IC_{50} values for E2020 against isoform-selective substrates for CYP450s 1A2, 2C9, 2C19, 2D6, and 3A4 are all in excess of 100 μM (>40 μg/mL) or more than 650 times the average plasma concentrations observed in patients receiving 10 mg/day in controlled clinical trials. Additional experiments characterized the Ki values for the interaction of E2020 with CYP2D6 and CYP3A4; mean Ki values for three individual sets of human liver microsomes were 47 μM (approximately 20 μg/mL) and 131 μM (approximately 55 μg/mL), respectively. Since the steady state Cmax of E2020 following 5 and 10 mg daily doses was found to be 31 ng/mL (82 nM) and 62 ng/mL (164 nM), respectively, the Ki values of E2020 for isozymes CYP 2D6 and CYP 3A4 were estimated to be at least 280 fold and 800 fold higher than the highest anticipated therapeutic concentrations of E2020 . This indic ates that drug-drug interactions of E2020 with other drugs that are metabolized by CYP2D6 ad CYP3A4 may not be anticipated as a consequence of coadministration of E2020.

In another *in vitro* study (Study 19952805), specific inhibitors of CYP2D6 (quinidine at 1-100 μM) were shown to inhibit the metabolism of E2020 (50 μM) by 58-77% (i.e. inhibit the formation of metabolites M1 and M2). Similarly, specific inhibitors of CYP 3A4 (ketoconazole at 0.2-2 μM) were shown to inhibit the metabolism of E2020 (50 μM) by 62-92% (i.e. inhibit formation of metabolite M4). These results suggest that the metabolism of E2020 is mainly mediated by CYP2D6 and CYP3A4.

D. Elimination:
E2020 and its metabolites are eliminated primarily via urinary excretion. Following a single 5 mg oral dose of 14C-E2020 to 8 healthy males, 57 ± 4% and 15 ± 6% of the radioactivity was recovered in urine and feces, respectively, over a period of 10 days, for a mean combined recovery of 72%. About 17% of the E2020 dose was recovered in the urine as unchanged drug and metabolites accounted for about 18% (metabolites M1, M2 and their conjugates M11 and M12 accounted for about 1.5%, 1%, 5%, and 2%, respectively; metabolite M4 for about 6% and metabolite M6 for about 2%); whereas about 22% of the dose recovered in urine was considered as unidentified metabolites. In feces, E2020 accounted for about 1% of the dose, metabolites M1, M2, M11, M12, M4, and M6 accounted for about 2%, while the remainder 12% of the dose recovered in feces is unidentified metabolites. The elimination of total radioactivity in the plasma was described by a biexponential equation (two compartment model) with a long terminal half-life of about 70 hours (CV = 18%). The mean apparent plasma clearance (Cl/F) of E2020 was found to be 0.13 L/hr/kg (Study E2020-A001-004).

III. DOSE PROPORTIONALITY:

In a multiple dose study involving 14 healthy male and female subjects, dose proportionality at two dose levels of 5 and 10 mg was evaluated (E2020-A001-011) and mean AUC^{ss} and $Cmax^{ss}$ values were found to be proportional to the dose. A similar finding was observed following administration of 1, 3, and 5 mg doses once daily for 21 days in 24 healthy male subjects (Study E2020-A001-002). Thus, overall it can be concluded that E2020 displays linear kinetics over the multiple dose range of 1-10 mg.

IV. MULTIPLE DOSE STUDY

Following oral administration of 5 and 10 mg doses once daily for 28 and 21 days, respectively, in 14 healthy male and female subjects, the mean steady-state $Cmax^{ss}$ and AUC^{ss} were 34 ng/mL (CV = 21%) and 61 ng/mL (CV = 17%); and 635 ng*hr/mL (CV = 15%) and 1128 ng*hr/mL (CV = 17%), respectively (Study E2020-A001-011). Steady-state was observed to be reached within 15 days. Upon multiple dose administration, E2020 accumulates in plasma. Following 28 and 21 days of administration of 5 and 10 mg doses once daily, respectively, an accumulation ratio of about 4 for Cmax and about 7 for AUC was observed. A similar finding was observed when 5 and 10 mg doses were administered for 28 days and 21 days, respectively (Study E2020-A001-003) and also following administration of 1, 3, and 5 mg doses for 21 days (Study E2020-A001-002).

Further, concentrations of E2020 were measured in cerebrospinal fluid (CSF) prior to final dose on day 28 and continued for another 48 hours. Steady-state CSF trough concentrations appear to be proportional to dose and CSF:Plasma E2020 concentration ratio was 16% (E2020-A001-003).

V. CHRONOPHARMACOKINETICS

The sponsor did not conduct a formal pharmacokinetic study to examine the chronopharmacokinetics of E2020. However, a retrospective analysis of pharmacokinetic data across studies was performed by this reviewer to identify time of administration on the pharmacokinetics of E2020. Comparable steady-state pharmacokinetic parameters were observed for 5 mg doses given to healthy volunteers in the morning for 21 days (E2020-A001-002) and in the evening for 28 days (E2020-A001-011), indicating no affect of time of administration (morning vs evening dose) on the pharmacokinetics of E2020.

VI. SPECIAL POPULATIONS:

Hepatic Impairment:

In a single-dose (5 mg), non-randomized, open label study involving 10 patients with stable alcoholic cirrhosis and 10 healthy, age and sex-matched subjects, the pharmacokinetics of E2020 was investigated (Study E2020-A001-005). Cmax and $AUC_{0-\infty}$ were found to be increased in hepatically impaired patients by 38% and 20%, respectively, while Tmax decreased by 33% in comparison to age and sex-matched healthy subjects. Only the difference in Cmax was found to be statistically significant ($P=0.02$). Based on these results, it can be concluded that hepatic impairment did not affect the pharmacokinetics of E2020.

Renal Impairment:

In a single-dose (5 mg), open label study involving 4 renal patients (creatine clearance (Cl_{cr})≤ 22 mL/min/1.73m^2) and 4 age and sex matched healthy subjects (Cl_{cr} ≥75 mL/min/1.73m^2), pharmacokinetics of E2020 was investigated (Study E2020-E044-001). The sponsor claimed this as an interim report, as the original protocol was designed to include 12 patients and 12 healthy subjects in the study. No differences in Cmax and Tmax, while an increase of 13% in AUC_{0-t} was observed in renally impaired patients in comparison to healthy subjects. Thus, it can be concluded that renal impairment did not affect the pharmacokinetics of E2020.

Elderly:

The sponsor did not conduct a formal pharmacokinetic study to examine age related differences in the pharmacokinetics of E2020. However, a retrospective analysis of pharmacokinetic data across studies was performed by this reviewer to identify age related differences in the pharmacokinetics of E2020.

In one study, the pharmacokinetics of E2020 was investigated in 6 healthy elderly Japanese subjects (mean age = 74 ± 8 years) following a single oral administration of 2 mg tablet after breakfast (Study E2020-J081-004). The results of this study were compared with another study where the pharmacokinetics of E2020 was evaluated in 6 healthy young Japanese subjects (mean age = 23 ± 2 years) following a single oral administration of 2 mg tablet after breakfast (Study E2020-J081-002). No significant differences in AUC and Cmax were observed in elderly in comparison to young healthy Japanese

subjects. Therefore, it can be concluded that age does not appear to affect the pharmacokinetics of E2020.

Gender:

The sponsor did not conduct a formal pharmacokinetic study to examine gender related differences in the pharmacokinetics of E2020. However, a retrospective analysis of pharmacokinetic data was performed by this reviewer to identify gender related differences in the pharmacokinetics of E2020.

In one study, the pharmacokinetics of E2020 was investigated in 8 healthy female subjects (mean body weight = 62 ± 5 Kg) and 7 healthy male subjects (mean body weight = 76 ± 6 Kg) following a single oral administration of 5 mg tablet (Study E2020-A001-008). On an average, females had 15% higher AUC values than males, which could be attributed to differences in body weight. Thus, gender does not appear to affect the pharmacokinetics of E2020.

Effect of Race:

The sponsor did not conduct a formal pharmacokinetic study to examine the affect of race on the pharmacokinetics of E2020. However, a retrospective analysis of pharmacokinetic data across studies conducted in Japanese (Study E2020-J081-001) and Caucasians (Study E2020-A001-008) was performed by this reviewer to identify the affect of race on the pharmacokinetics of E2020.

Comparison of the pharmacokinetics between Japanese and Caucasians at the proposed labeled dosing regimen of 5 mg once a day, revealed no significant differences in AUC or Clearance.

VII. PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIPS

The sponsor has evaluated the pharmacokinetic and pharmacodynamic relationship of E2020 following multiple dose administration of 5 and 10 mg tablets (Study E2020-A001-011). The sponsor has used rbc-cholinesterase inhibition as the surrogate marker, since Alzheimer's disease is associated with a relative decrease in cholinergic activity and E2020 presumably exerts its action by inhibition of AChE. Inhibition of AChE is related to plasma concentrations by the E_{max} model.

AchE Inhibition % = E_{max} * E2020/(EC_{50} + E2020)

where: E_{max} = maximal percent inhibition of AChE
EC_{50} = concentration of E2020 that diminishes the activity of AchE by 50%

A significant relationship was observed between E2020 plasma concentrations and the percent acetylcholinesterase inhibition (AChEI). This relationship was consistent during each of the 24 hour periods evaluated on Days 1, 7, 14, 21 and 28, and demonstrates that there are no time dependent changes in E2020 pharmacodynamics. Area under the effect curve for AChE inhibition versus concentration for each of the five 24-hour characterizations were found to be superimposable. Mean steady state plasma concentrations of 34 ng/mL (CV = 21%) and 61 ng/L (CV = 17%) for the 5 mg/day and 10 mg/day doses, resulted in a mean steady state AChE inhibition (E_{ss}%) of 65 ± 5% and 78 ± 3%, respectively. A similar finding was observed in clinical trials (E2020-A001-301 and 302) indicating that the inhibition of AChE is comparable between healthy subjects and patients, and remains stable over time. However, the sponsor was unable to develop a meaningful relationship between AChE inhibition and clinical end point such as ADAS-COG score (a measure of cognitive function).

VIII. DRUG INTERACTIONS

Cimetidine: In a randomized, open label, three period, multiple dose, cross-over drug interaction study between E2020 and cimetidine involving 18 healthy male subjects, the pharmacokinetics of E2020 and cimetidine was investigated alone and in combination following oral administration of 5 mg of E2020 and 800 mg cimetidine (Study E2020-A001-006). It was observed that the concurrent administration of E2020 and cimetidine resulted in increased plasma levels of E2020 as reflected by statistically significant increases in Cmax (13%) and AUC_{0-24} (12% , both Day 1 and Day 7, but may not have clinical significance. No such differences were observed in the pharmacokinetic parameters of cimetidine, when administered alone or in combination. Further, the accumulation ratio was found to be the same for both E2020 and cimetidine upon multiple dose administration either alone or in combination. Therefore, it can be concluded that cimetidine does not affect the pharmacokinetics of E2020 and vice versa.

Digoxin: In a randomized, open label, three period, single dose, cross-over drug interaction study between E2020 and digoxin involving 12 healthy male subjects, the pharmacokinetics of E2020 and digoxin was investigated alone and in combination following oral administration of 5 mg of E2020 and 0.25 mg

of digoxin (Study E2020-A001-007). No statistically significant differences were observed in the pharmacokinetic parameters (e.g., AUC, Cmax, Tmax or Half-life) of E2020 and digoxin when coadministered. Thus, it can be concluded that digoxin does not affect the pharmacokinetics of E2020 and vice versa.

Theophylline: In a randomized, two-period, open label, multiple dose, cross-over drug interaction study between E2020 (once daily) and sustained release formulation of theophylline (twice daily) involving 12 healthy male subjects, the pharmacokinetics of theophylline was investigated alone and in combination with E2020 following oral administration of appropriate theophylline dose to achieve therapeutic concentrations (10-20 μg/mL) and 5 mg of E2020 (Study E2020-E044-002). No statistically significant differences in the pharmacokinetics of theophylline were observed in the presence of E2020. Thus, it can be concluded that E2020 did not affect the pharmacokinetics of theophylline.

Warfarin: In a randomized, balanced, two-period, open label, multiple dose, cross-over drug interaction study between E2020 and warfarin involving 12 healthy male subjects, the pharmacokinetics of (R)-warfarin and (S)-warfarin was investigated alone and in combination with E2020 following oral administration of a single 25 mg dose of warfarin and multiple doses of 10 mg of E2020 once daily (Study E2020-E044-003). No statistically significant differences in the pharmacokinetics and pharmacodynamics (prothrombin time) of (R)-warfarin and (S)-warfarin were observed in the presence of E2020. Thus, it can be concluded that E2020 did not affect the pharmacokinetics or pharmacodynamics of warfarin. However, subject 5 showed an unusually high values for AUC and half-life for (S)-warfarin, in both treatments i.e., with and without E2020, probably due to metabolic differences. But no such observation was made for (R)-warfarin.

IX. PLASMA PROTEIN BINDING INTERACTIONS

E2020 is highly bound (96%) to plasma proteins (Study 19952677). Drug displacement studies have been performed *in vitro* between this highly bound drug and other drugs such as furosemide, digoxin, and warfarin (Study E2020-950119). E2020 at concentrations of 0.3-10 μg/mL did not affect the binding of furosemide (5 μg/mL), digoxin (2 ng/mL), and warfarin (3 μg/mL) to human albumin. Similarly the binding of E2020 to human albumin was not affected by furosemide, digoxin and warfarin.

APPENDIX II: ANALYTICAL METHODOLOGY

E2020 was quantified in plasma, whole blood, urine, and feces by In plasma and urine E2020 had a limit of quantitation Overall, the analytical validation was found to be satisfactory in terms of specificity, sensitivity, linearity, precision, and accuracy. An equilibrium dialysis method was used to support protein binding determinations.

APPENDIX III: DRUG FORMULATION

E2020 tablet formulations used in the clinical trials/pharmacokinetic studies, and the proposed to be marketed formulation are identical with the exception of minor changes such as:

1. Excipient specifications
2. and
3.

APPENDIX IV: IN VITRO DISSOLUTION

The sponsor provided *in vitro* dissolution information of film-coated E2020 tablets (bio-batch; the to-be-marketed dosage form) at 1-2 time points (15 and/or 30 minutes) From the data it was observed that dissolution for the film-coated tablets is rapid in (greater than dissolved in 15 minutes).

The sponsor was requested to provide dissolution profiles, both graphical as well as tabular data of 12 individual tablets and mean data, at least in 3 media However, based on the results provided, an "interim" dissolution methodology and specification is recommended for Aricept 5 mg film-coated tablets as follows:

Medium:
Apparatus:
Specification:

Comments To Be Sent To The Firm:

1) The concentrations of Aricept used in *in vitro* studies are so high (10 μM) that switchability from a high affinity, low capacity enzyme (e.g., CYP 2D6) to a low affinity, high capacity enzyme (e.g., CYP 3A4) is a clear possibility. Therefore, conducting *in vitro* studies using relevant therapeutic concentrations of Aricept (≤164nM) may be useful to determine the specific isozyme responsible for the metabolism of Aricept. Further, *in vivo* studies to investigate the effect of specific inhibitors of CYP 2D6 (e.g., paroxetine, fluoxetine, and quinidine) and CYP 3A4 (e.g., ketoconazole, clotrimazole, and ethinylestradiol) on the metabolism of Aricept could be more conclusive. Because CYP 2D6 displays a genetic polymorphism (8% of Caucasians are classified as poor metabolizers), the sponsor may consider ascertaining whether people deficient in this enzyme have an impaired ability to metabolize E2020.

2) The sponsor has provided AUC_{0-} values for total radioactivity and E2020 in plasma. However, in order to determine the amount of exposure to metabolites, it is important to know their individual AUC_{0-} values.

3) In future food effect studies, the sponsor is requested to use the highest strength of the to be marketed dosage form and FDA recommended high fat meal.

4) The sponsor is encouraged to further develop the analytical method so that it is sensitive enough to characterize the pharmacokinetic profile of the parent compound for at least 5 half-lives, as opposed to characterization for only 2-3 half-lives.

5) In the bioequivalence studies, 90% confidence interval analysis should be performed using log transformed data for AUC_{0-}, instead of using only untransformed data.

6) The sponsor is requested to submit the study reports of the population pharmacokinetic analysis including the control files to evaluate the effect of population covariates on the pharmacokinetics of E2020.

7) The sponsor is requested to submit the composition of the formulations used in biostudies (Kawashima, Lot # 46016ZZZ, 41016ZZA; AAI, Lot # 95025C; Pfizer Inc., Lot # N5111) and at least three representative lots from clinical trials 301 (e.g., Lot #s: K3Y002ZZZ, K3X015ZZZ, and K42009ZZZ) and 302 (e.g., Lot #s: K3Y003ZZZ, K37017ZZA, and K42016ZZZ).

8) The sponsor is requested to submit dissolution profiles, both graphical as well as tabular data of 12 individual tablets and mean data, for each strength of Aricept tablets manufactured at both the sites and Pfizer in 3 media
This will provide the necessary dissolution data to support the biowaiver request for the 10 mg tablet and also allow for setting a more appropriate dissolution specification for both the 5 and 10 mg tablets.

9) The sponsor is requested to adopt the following "interim" dissolution methodology and specification for Aricept 5 mg film-coated tablet:

Medium:
Apparatus:
Specification:

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LABELING COMMENTS

The firm is requested to perform the following revisions on the submitted annotated draft labeling:

1. The bioavailability and pharmacokinetic information for **ARICEPT™** tablets provided in the Pharmacokinetics portion of the Clinical Pharmacology section should be replaced with the following:

CLINICAL PHARMACOLOGY: PHARMACOKINETICS

Absorption and Distribution:

Aricept™ is well absorbed with a relative oral bioavailability of and reaches peak plasma concentrations in 3 to 4 hours. Pharmacokinetics are linear over a dose range of 1-10 mg given once daily. Neither food nor time of administration (morning vs evening dose) appear to have influence on the rate and extent of Aricept absorption. Upon multiple dose administration, Aricept accumulates in plasma by 4-7 fold and steady state is reached within 15 days.

The steady-state volume of distribution is 12 L/kg (CV = 17%). Aricept™ is approximately 96% bound to human plasma proteins mainly to albumins (about 75%) and α_1-acid glycoprotein (about 21%) over the concentration range of The distribution of Aricept™ in various body tissues has not been

definitively studied.

Metabolism and Elimination:

Aricept™ is both excreted in the urine intact and extensively metabolized to four major and a number of minor metabolites, not all of which have been identified. The metabolism of Aricept consists of CYP 450 dependent oxidation and glucuronidation. *In vitro* experiments using human liver microsomes demonstrated that CYP2D6 and CYP3A4 are the principal isoenzymes involved in the metabolism of Aricept. Following administration of a single 5 mg dose of ^{14}C-labeled Aricept, plasma radioactivity, expressed as a percent of the administered dose, was present primarily as intact Aricept (53%) and as 6-O-desmethyl donepezil (11%), which has been reported to inhibit AChE to the same extent as Aricept *in vitro* and was found to be present in plasma at concentrations equal to about 20% of Aricept. Approximately 57% and 15% of the total radioactivity was recovered in urine and feces, respectively, over a period of 10 days, for a mean combined recovery of 72%, while 28% remained unrecovered. This suggests that Aricept™ and/or one of its metabolites may persist in the body for more than 10 days. About 17% of the Aricept dose was recovered in the urine as unchanged drug, and metabolites accounted for about 18%; whereas about 22% of the dose recovered in urine was considered as unidentified metabolites. In feces, Aricept accounted for about 1% of the dose, metabolites accounted for about 2%, while the remainder 12% of the dose recovered in feces is unidentified metabolites. The mean apparent plasma clearance (Cl/F) of Aricept was found to be 0.13 L/hr/kg. The mean elimination half-life is approximately 70 hours (CV = 18%) after repeated doses.

The pharmacokinetic and pharmacodynamic relationship established by Emax model indicated a significant relationship between Aricept plasma concentrations and the percent inhibition of AChE and demonstrated no time dependent changes in Aricept pharmacodynamics. However, such a relationship was not observed between AChE inhibition and clinical end point such ADAS-COG score (a measure of cognitive function). The inhibition of AChE is comparable between healthy subjects and patients, and remains stable over time. The minimum, maximum and steady-state plasma concentrations (C) and pharmacodynamic effect (E, percent inhibition of acetylcholinesterase in erythrocyte membranes) of Aricept™ are given in Table 1.

Table 1. Plasma Concentrations and Pharmacodynamic Effect of ARICEPT™ at Steady-state (Mean ± S.D.).

Dose (mg/day)	C_{min} (ng/mL)	C_{max} (ng/mL)	C_{ss} (ng/mL)	E_{min} (%)	E_{max} (%)	E_{ss} (%)
5	21 ± 4	34 ± 7	27 ± 4	62 ± 6	72 ± 4	65 ± 5
10	39 ± 9	61 ± 10	47 ± 8	75 ± 4	84 ± 2	78 ± 3

Special Populations:

Hepatic Disease : Clearance of Aricept™ is decreased by 20% in patients with hepatic disease, which was found not to be statistically significant.

Renal Disease: Clearance of Aricept™ did not change in patients with moderate to severe renal impairment (Cl_{cr} < 22 mL/min/1.73 m^2).

Age: No formal pharmacokinetic study was conducted to examine age related differences in the pharmacokinetics of Aricept. However, mean plasma Aricept™ concentrations measured during therapeutic drug monitoring of elderly patients with Alzheimer's Disease are comparable to those observed in young healthy volunteers.

Gender and Race: No specific pharmacokinetic study was conducted to investigate the effect of gender and race on the disposition of Aricept. However, retrospective pharmacokinetic analysis indicate that gender and race (Japanese and Caucasians) did not affect the clearance of Aricept™.

2. The following information should be included under the "Drug interactions" section of the proposed labeling:

Drug-Drug Interactions

Drugs Highly Bound to Plasma Proteins:

Drug displacement studies have been performed *in vitro* between this highly bound drug (96%) and other drugs such as furosemide, digoxin, and warfarin. Aricept at concentrations of 0.3-10 μg/mL did not affect the binding of furosemide (5 μg/mL), digoxin (2 ng/mL), and warfarin (3 μg/mL) to human

albumin. Similarly, the binding of Aricept to human albumin was not affected by furosemide, digoxin and warfarin.

Effect of Aricept on the Metabolism of Other Drugs:

In vitro experiments using human liver microsomes demonstrated that CYP2D6 and CYP3A4 are the principal isoenzymes involved in Aricept metabolism. Mean Ki values for the interaction of Aricept with isozymes CYP 2D6 and CYP 3A4 were estimated to be 47 μM and 131 μM, respectively, and were found to be at least 280-fold and 800-fold higher than the highest anticipated therapeutic concentrations (61 ng/mL i.e., 164 nM) of Aricept at steady-state. This suggests that drug-drug interaction potential of Aricept with other drugs that are metabolized by CYP2D6 and CYP3A4 may not be anticipated. Currently, it is not known whether Aricept has any potential for enzyme induction.

Formal pharmacokinetic studies evaluated the potential of Aricept for interaction with theophylline, cimetidine, warfarin and digoxin. No significant effects on the pharmacokinetics of these drugs were observed.

Effect of Other Drugs on the Metabolism of Aricept:

In vivo studies demonstrated that the metabolism of Aricept is not significantly affected by concurrent administration of digoxin or cimetidine. However, potential interactions may occur when Aricept is given concurrently with agents that affect CYP 2D6 and CYP 3A4 activity. Potential inhibitors of CYP 2D6 (e.g., fluoxetine and quinidine) and CYP 3A4 (e.g., ketoconazole, clotrimazole, and ethinylestradiol) could decrease the rate of Aricept elimination; while inducers of CYP 2D6 and CYP 3A4 (e.g., phenytoin, carbamazepine, daxamethasone, rifampin, and phenobarbital) could increase the rate of elimination of Aricept.

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08/01/96

-- Vijay K. Tammara, Ph. D.
Division of Pharmaceutical Evaluation I

First Draft prepared on July 14, 1996
RD Initialed by M. Hossain, Ph. D. On July 16, 1996

Second Draft prepared on July 19, 1996
RD Initialed by M. Hossain, Ph. D. On July 20, 1996
Third Draft prepared on July July 24, 1996
RD Initialed by M. Hossain, Ph. D. On July 24, 1996

Biopharm Day: 07/30/1996 (2 pm-3:30 pm) (Attendees: Hank Malinowski, Mehul Mehta, Jerry Collins, Nicholas Fleischer, John Hunt, Mohammad Hossain, Vijay Tammara, Randy Levin, Glenna Fitzgerald, Barry Rosloff, and Katurah Higgins).

FT Initialed by M. Hossain, Ph. D. M.K. Hossain 8/1/96

CC: NDA 20,690 (orig.), HFD-120, HFD-860 (Tammara, Hossain, Malinowski), HFD-340 (Vish), HFD-019, DI, PK/PD, F, E, G, A, HFD-870 (Clarence Bott (Rm 13B31 PKLN): Drug, Chron, Division, and Reviewer Files).

APPENDIX I

RELATIVE ORAL BIOAVAILABILI

CLINICAL STUDY SYNOPSIS

Title: A Study of the Relative Bioavailability of E2020 in Healthy Volunteers; Study #: E2020-A001-008

Investigator:

Objective: The objective of this study was to determine the bioavailability of the 5-mg E2020 clinical trials tablet formulation relative to an aqueous solution containing 5-mg of E2020, following the administration of single, oral doses.

Study Design: This study was a randomized, single-dose, open label, two-period crossover study which was conducted at a single investigational site.

Study Population: A total of 16 healthy volunteers were randomized into this study and all 16 subjects completed the study without incident. The study population was comprised of 8 males and 8 females. Participants were Caucasion (14), Hispanic (1) and Asian (1), and ranged in age from 22 to 63 years (mean ± SD: 45.8 ± 3.2 years) and in weight from 55.1 to 84.2 kg (mean ± SD: 68.9 ± 2.2 kg).

Treatment Groups: The two treatments administered in this study were: 1) E2020, 5-mg tablet; and 2) E2020, 5-mg aqueous solution. Medication was administered after a fast of at least eight hours and was accompanied by a total of 250 ml of tap water.

Methods *(Clinical)*: Volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to the start of the study. For each Treatment period, subjects were admitted to the study site on the evening of day 0, at least 12 hours prior to drug administration. Upon admission, a routine physical examination was conducted. Entry vital signs (sitting position), and weight were recorded, and urine specimens for drug use/abuse screening were collected. A light snack was served 10 hours prior to drug administration, after which an absolute fast, from both food and fluids (except water), was maintained. The following morning, prior to drug administration; clinical laboratory and analytical samples were collected. Following drug administration, blood samples for analytical determinations were collected at specified intervals for 24 hours. Subjects were then discharged from the study site the following morning, after providing a 24-hour blood sample. Volunteers then returned to the study site at 24-hour intervals (to 120 hours), for additional analytical sample collection. Total in-house time for each treatment period was approximately 36 hours.

Methods *(Analytical)*: E2020 concentrations in plasma were determined by . The limits of quantitation for the assay were E2020 pharmacokinetics were characterized using the resultant analytical data and standard techniques. Parameters used for between-treatment comparisons included C_{max}, t_{max}, $t_{1/2}$, AUC_{0-120}, and $AUC_{0-\infty}$. The relative bioavailability (F_{rel} (%)) was calculated as 100 x $AUC_{0-\infty}$ tablet / $AUC_{0-\infty}$, solution.

Results:

Pharmacokinetics: The pharmacokinetic profiles of the E2020 5-mg tablet and the E2020 5-mg solution were virtually identical. There was no statistically significant difference between the tablet group and the solution group in any of the PK parameters examined. The mean relative bioavailability F_{rel} (%) was calculated to be 101.3% ± 3.07%.

A summary table containing several of the PK parameters calculated is presented below:

Comparison of Pharmacokinetic Parameters (Mean ± SE)

	C_{max} (ng/ml)	t_{max} (hrs)	$t_{1/2}$ (hrs)	AUC (0-120) (ng*hr/ml)	AUC (0-∞) (ng*hr/ml)
E2020 tablet	7.3 (0.4)	4.7 (0.4)	77.9 (5.8)	356.5 (22.8)	498.0 (35.1)
E2020 aqueous solution	7.4 (0.3)	4.7 (0.4)	77.6 (5.0)	352.0 (20.6)	492.5 (30.6)
P-value	0.337	0.769	0.907	0.677	0.821

Safety: No serious or unexpected adverse experiences occurred during the course of this study. Treatment Emergent Signs and Symptoms (TESS) were transient and all were mild or moderate in severity.

No clinically significant, treatment emergent abnormal laboratory values (TEAV's) were observed during the course of this study.

Conclusions:

The conclusion from this study is:

- The bioavailability of the E2020 5-mg tablet formulation is approximately 100% relative to the E2020 5-mg aqueous solution formulation.

Eisai America, Inc.
Protocol: E2020-A001-008
Investigator:

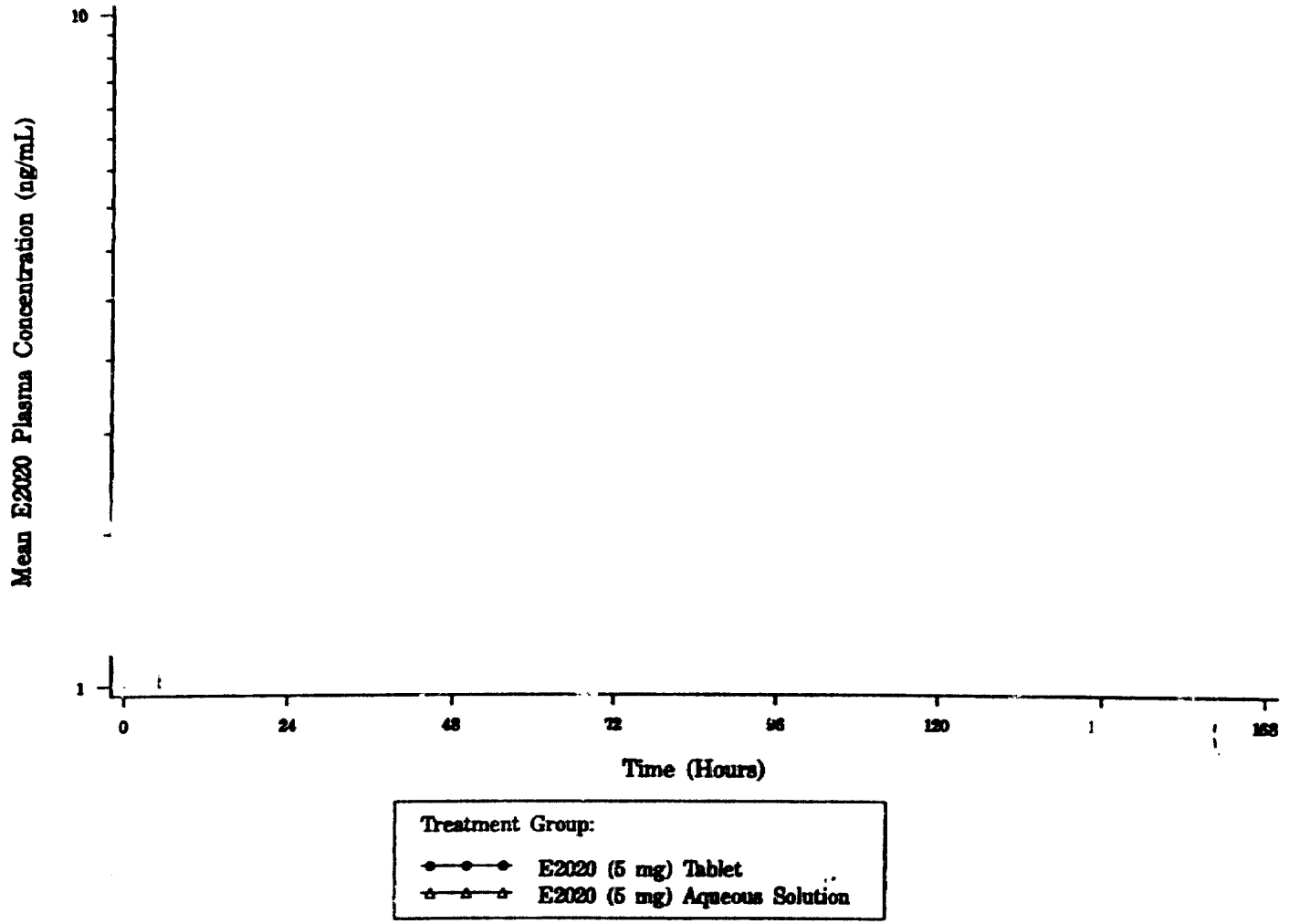
Table 8
Pharmacokinetic Parameters

		E2020 5mg Tablet	E2020 5mg Solution	P-value
$AUC_{0\text{-}120\ hrs}$ (ng*hr/mL)				
	N	*15*	*15*	
	Mean (S.E.)	*356.5 (22.8)*	*352.0 (20.6)*	*0.6774*
	Min - Max	*195.9 - 544.8*	*225.2 - 474.5*	
$AUC_{0-\infty}$ (ng*hr/mL)				
	N	*15*	*15*	
	Mean (S.E.)	*498.0 (35.1)*	*492.5 (30.6)*	*0.8210*
	Min - Max	*314.4 - 839.3*	*326.1 - 703.1*	
t_{max} (hour)				
	N	*15*	*15*	
	Mean (S.E.)	*4.7 (0.4)*	*4.7 (0.4)*	*0.7690*
	Min - Max	*3.0 - 8.0*	*3.0 - 8.0*	
C_{max} (ng/mL)				
	N	*15*	*15*	
	Mean (S.E.)	*7.3 (0.4)*	*7.4 (0.3)*	*0.3375*
	Min - Max	*4.8 - 10.0*	*5.0 - 9.6*	
λ_z (hour^{-1})				
	N	*15*	*15*	
	Mean (S.E.)	*0.010 (.001)*	*0.009 (.001)*	*0.5137*
	Min - Max	*0.006 - 0.016*	*0.006 - 0.014*	
$t_{1/2}$ (hour)				
	N	*15*	*15*	
	Mean (S.E.)	*77.9 (5.8)*	*77.6 (5.0)*	*0.9079*
	Min - Max	*43.5 - 119.4*	*50.1 - 114.9*	
Cl_T/F (L/hour/kg)				
	N	*15*	*15*	
	Mean (S.E.)	*0.158 (.010)*	*0.158 (.010)*	*0.9570*
	Min - Max	*0.086 - 0.229*	*0.103 - 0.221*	
$V_{\lambda z}/F$ (L/kg)				
	N	*15*	*15*	
	Mean (S.E.)	*16.78 (.864)*	*16.87 (.669)*	*0.7585*
	Min - Max	*12.01 - 22.76*	*13.84 - 20.06*	
F_{rel} (%)				
	N	*15* /2.0		
	Mean (S.E.)	*101.3 (2.67)*		
	Min - Max	*80.71 - 126.0*		

Data Source: Statistical Appendix VII

Figure II
E2020 – A001 – 008

E2020 Plasma Concentration (ng/mL)



Statistical Appendix VII
E2020 Pharmacokinetic Parameters by Subject

DOSE GROUP	SUBJECT NUMBER	WEIGHT (KG)	SEQUENCE	Cmax (ng/mL)	tmax (hrs.)	AUC(0-120) (ng hr/mL)	AUC(0-inf.) (ng hr/mL)	CL/F (L/hr/kg)	HALF LIFE (hrs.)	ELIMINATION RATE CONSTANT(K) (hour^-1)	V/F (L/kg)	Frel(%
E2020 5mg solution	001											
	002											
	003											
	004											
	005											
	006											
	008											
	009											
	010											
	011											
	012											
	013											
	014											
	015											
	016											
E2020 5mg tablet	001											
	002											
	003											
	004											
	005											
	006											
	008											
	009											
	010											
	011											
	012											
	013											
	014											
	015											
	01.											

BIOEQUIVALENCE STUDY CAVSR

CLINICAL STUDY SYNOPSIS

Title: A Study of the Bioequivalence of Three Tablet Formulations of E2020, in Healthy Volunteers; Study #: E2020-A001-009

Investigator:

Objective: The objective of this study was to determine the bioequivalence of two different 5-mg E2020 tablet formulations relative to the E2020 reference 5-mg tablet formulation used in all clinical trials.

Study Design: This study was a randomized, single-dose, open label, three-period crossover study which was conducted at a single investigational site. Each treatment period was followed by a 2-week washout period.

Study Population: Volunteers for this study were healthy, ambulatory, non-smoking male volunteers, aged 18-45 years. A total of 19 volunteers were randomized into this study and 18 completed the study without incident. All participants were Caucasion and ranged in age from 19 to 45 years and in weight from 63.9 to 85.2 kg.

Treatment Groups: Subjects randomly received each of the three following E2020 formulations:

Formulation A: 5.0-mg, film-coated E2020 tablets produced by Eisai Company, Ltd. in Kawashima, Japan. This is the REFERENCE formulation. Lot # K46016ZZZ

Formulation B: 5.0-mg, film-coated tablets produced by Lot # 95025C

Formulation C: 5.0-mg, film-coated tablets produced by Lot # 99278

Medication was administered after a fast of at least eight hours and was accompanied by a total of 250-ml of tap water.

Methods *(Clinical)*: Volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to the start of the study. For each Treatment period, subjects were admitted to the study site on the evening of day 0, at least 12 hours prior to drug administration. Upon admission, a routine physical examination was conducted. Entry vital signs (sitting position), and weight were recorded, and urine specimens for drug use/abuse screening were collected. A light snack was served 10 hours prior to drug administration, after which

an absolute fast, from both food and fluids (except water), was maintained. The following morning, prior to drug administration, clinical laboratory and analytical samples were collected. During each treatment period subjects received a single 5-mg tablet of E2020. The order of formulation administration was randomized. Following drug administration, blood samples for analytical determinations were collected at specified intervals for 24 hours. Subjects were then discharged from the study site the following morning, after providing a 24-hour blood sample. Volunteers then returned to the study site at 24-hour intervals (to 168 hours), for additional analytical sample collection. Total in-house time for each treatment period was approximately 36 hours. Each treatment period (inpatient + outpatient) was 7 days in duration, and was follow by a 2-week washout period.

Methods *(Analytical)*: E2020 concentrations in plasma were determined by The limits of quantitation for the assay were E2020 pharmacokinetics were characterized using the resultant analytical data and standard techniques. Parameters used for between-treatment comparisons included C_{max}, Log_{10} C_{max}, t_{max}, AUC_{0-168}, Log_{10} AUC_{0-168}, $AUC_{0-\infty}$ and $t_{1/2}$.

Results: Pharmacokinetics: For the primary pharmacokinetic parameters AUC_{0-168} and C_{max}, both test formulations were found to be bioequivalent to the reference formulation (Kawashima) when using both the original data scale, as well as the log-transformed data. Similar results were observed for both t_{max} and $t_{1/2}$. All of the 90% Confidence Intervals for the ratio of test formulation/reference formulation were well within the 80% - 120% interval for the raw data, and within the 80% - 125% interval for the log transformed data.

The primary pharmacokinetic parameters are listed in the table below.

Comparison of Pharmacokinetic Parameters (Mean ± SE)

	C_{max} (ng/ml)	Log_{10} C_{max} (ng/ml)	$AUC_{(0-168)}$ (ng*hr/ml)	Log_{10} $AUC_{(0-168)}$ (ng*hr/ml)
Formulation A (Kawashima - Ref.)	7.4 (0.2)	0.866 (.012)	323.6 (19.4)	2.496 (.028)
Formulation B	7.5 (0.3)	0.871 (.017)	340.7 (24.7)	2.509 (.037)
Formulation C	7.3 (0.3)	0.856 (.017)	324.2 (19.2)	2.497 (0.27)

5.4 PHARMACOKINETICS

E2020 plasma concentrations for each individual subject are presented by blood sampling timepoint and E2020 formulation in DATA LISTING 16. Summary statistics for each timepoint and each E2020 formulation are presented in TABLE 7. A Time versus Concentration curve of the mean data (± SE) is presented in FIGURE II.

Subject #016 (discontinued secondary to acute pharyngitis) completed only 2 of the 3 periods and was not used in any of the pharmacokinetic (PK) analyses. This subject was replaced by subject #116. The complete results of the PK analyses and comparisons between groups are presented in TABLE 8. The primary PK parameters and 90% Confidence Intervals for the ratio of test/reference are summarized in the table below.

Comparison of Pharmacokinetic Parameters (Mean ± SE)

	C_{max} (ng/ml)	$Log_{10} C_{max}$ (ng/ml)	$AUC_{(0\text{-}168)}$ (ng*hr/ml)	$Log_{10} AUC_{(0\text{-}168)}$ (ng*hr/ml)	t_{max} (hrs)
Formulation A (Kawashima - Ref.)	7.4 (0.2)	0.866 (.012)	323.6 (19.4)	2.496 (.028)	4.2 (0.2)
Formulation B	7.5 (0.3)	0.871 (.017)	340.7 (24.7)	2.509 (.037)	4.2 (0.3)
Formulation C	7.3 (0.3)	0.856 (.017)	324.2 (19.2)	2.497 (0.27)	4.4 (0.4)
Ratio:	101.4%	100.6%	105.3%	100.5%	100.0%
90% C. I. for ratio: Test/Reference	97.7 - 106.2	97.1 - 105.7	100.8 - 109.7	98.5 - 107.8	87.7 - 112.3
Ratio:	98.6%	98.9%	100.2%	100.0%	104.8%
90% C. I. for ratio: Test/Reference	94.2 - 102.7	93.7 - 102.0	95.7 - 104.6	95.9 - 104.9	94.4 - 119.0

For the primary pharmacokinetic parameters $AUC_{(0\text{-}168)}$ and C_{max}, both test formulations were found to be bioequivalent to the reference formulation when using both the original data scale, as well as the log-transformed data. Similar results were observed for both t_{max} and $t_{1/2}$. All of the 90% Confidence Intervals for the ratio of test formulation/teference formulation for both primary parameters were well within the 80% - 120% interval for the raw data, and within the 80% - 125% interval for the log transformed data (see TABLE 8).

Individual bioequivalence for subjects receiving the formulation was observed in 17/18 subjects (94%) for AUC_{0-168}, and 16/18 subjects (89%) for C_{max}. For subjects receiving the formulations, individual bioequivalence was observed in 15/18 subjects (83%) for AUC_{0-168}, and 15/18 subjects (83%) for C_{max}.

Safety: No serious or unexpected adverse experiences occurred during the course of this study. Treatment Emergent Signs and Symptoms (TESS) were transient and all were mild or moderate in severity.

No clinically significant, treatment emergent abnormal laboratory values (TEAV's) were observed during the course of this study.

Conclusions:

The conclusion from this study is:

- Formulation B and Formulation C were both found to be bioequivalent to the reference formulation, Formulation A (Kawashima).

  For both test formulations bioequivalence relative to the reference formulation was clearly demonstrated in both mean bioavailability, as well as in bioavailability in individual subjects.

Eisai America, Inc.
Protocol: E2020-A001-009
Investigator:

Table 8
A Comparison of Pharmacokinetic Parameters

	E2020 5mg	E2020 5mg	KAWASHIMA[R] E2020 5mg	AAI vs. KAWASHIMA[R] 90% C.I. for Ratio* Test/Reference	AAI vs. KAWASHIMA[R] Two One-sided@ t-tests (T_U, T_L)	BOOTS vs. KAWASHIMA[R] 90% C.I. for Ratio* Test/Reference	BOOTS vs. KAWASHIMA[R] Two One-sided@ t-tests (T_U, T_L)
$AUC_{0\text{-}168\ hrs}$ (ng*hr/mL)							
N	18	18	18				
Mean (S.E.)	340.7 (24.7)	324.2 (19.2)	323.6 (19.4)	(100.9 , 109.7)	(-5.59 , 9.60)	(95.7 , 104.6)	(-7.52 , 7.67)
Min - Max	122.4 - 489.8	181.6 - 456.6	176.9 - 482.2				
Coeff. Var.	10.6%	11.1%	11.2%				
$Log_{10} AUC_{0\text{-}168\ hrs}$ (ng*hr/mL)							
N	18	18	18				
Mean (S.E.)	2.509 (.037)	2.497 (.077)	2.496 (.028)	(98.5 , 107.8)	(-7.28 , 9.55)	(95.9 , 104.9)	(-8.29 , 8.54)
Min - Max	2.088 - 2.690	2.259 - 2.660	2.247 - 2.683				
Coeff. Var.	0.074%	0.074%	0.074%				
Geometric Mean	322.7	314.1	313.1				
$AUC_{0-\infty}$ (ng*hr/mL)							
N	18	18	18				
Mean (S.E.)	454.9 (34.7)	452.0 (31.1)	437.4 (29.9)	(98.6 , 109.4)	(-4.99 , 7.48)	(97.9 , 108.8)	(-5.19 , 7.28)
Min - Max	167.2 - 769.7	279.1 - 741.7	249.8 - 759.8				
Coeff. Var.	13.1%	13.2%	13.6%				

Data Source: Statistical Appendix VII.

R = Kawashima is the reference formulation.

continued...

* 90% confidence interval on the ratio of test formulation to the reference formulation.

@ Schuirmann's Two one-sided tests:
Formulations are deemed bioequivalent if the classical (shortest) 90% Confidence Interval is within the 80-120% interval for the raw data, and within the 80-125% interval for the log-transformed data.
Bioequivalance is also declared if the absolute value of both t-tests are greater then 1.694

Eisai America, Inc.
Protocol: E2020-A001-009
Investigator

Table 8
A Comparison of Pharmacokinetic Parameters

	AAI E2020 5mg	BOOTS E2020 5mg	KAWASHIMA[R] E2020 5mg	AAI vs. KAWASHIMA[R] 90% C.I. for Ratio* Test/Reference	AAI vs. KAWASHIMA[R] Two One-sided@ t-tests (T_U, T_L)	BOOTS vs. KAWASHIMA[R] 90% C.I. for Ratio* Test/Reference	BOOTS vs. KAWASHIMA[R] Two One-sided@ t-tests (T_U, T_L)
C_{max} (ng/mL)							
N	18	18	18				
Mean (S.E.)	7.5 (0.3)	7.3 (0.3)	7.4 (0.2)	(97.7 , 106.2)	(-7.14 , 8.68)	(94.2 , 102.7)	(-8.52 , 7.10)
Min - Max	5.7 - 10.0	5.4 - 10.0	5.6 - 8.6				
Coeff. Var.	10.5%	10.9%	10.7%				
$Log_{10}C_{max}$ (ng/mL)							
N	18	18	18				
Mean (S.E.)	0.871 (.017)	0.856 (.017)	0.866 (.012)	(97.1 , 105.7)	(-8.43 , 9.47)	(93.7 , 102.0)	(-9.85 , 8.05)
Min - Max	0.758 - 1.002	0.734 - 1.001	0.749 - 0.933				
Coeff. Var.	0.070%	0.070%	0.070%				
Geometric Mean	7.4	7.2	7.3				
t_{max} (Hour)							
N	18	18	18				
Mean (S.E.)	4.2 (0.3)	4.4 (0.4)	4.2 (0.2)	(87.7 , 112.3)	(-2.75 , 2.75)	(94.4 , 119.0)	(-1.83 , 3.66)
Min - Max	2.0 - 6.0	3.0 - 8.0	3.0 - 6.0				
Coeff. Var.	30.9%	29.0%	30.9%				
$t_{1/2}$ (Hour)							
N	18	18	18				
Mean (S.E.)	63.23 (5.45)	65.64 (6.26)	61.75 (4.58)	(95.5 , 109.3)	(-4.30 , 5.47)	(99.4 , 113.1)	(-3.34 , 6.42)
Min - Max	26.31 - 116.3	39.62 - 96.99	34.19 - 111.2				
Coeff. Var.	17.0%	16.4%	17.4%				

Data Source: Statistical Appendix VII.

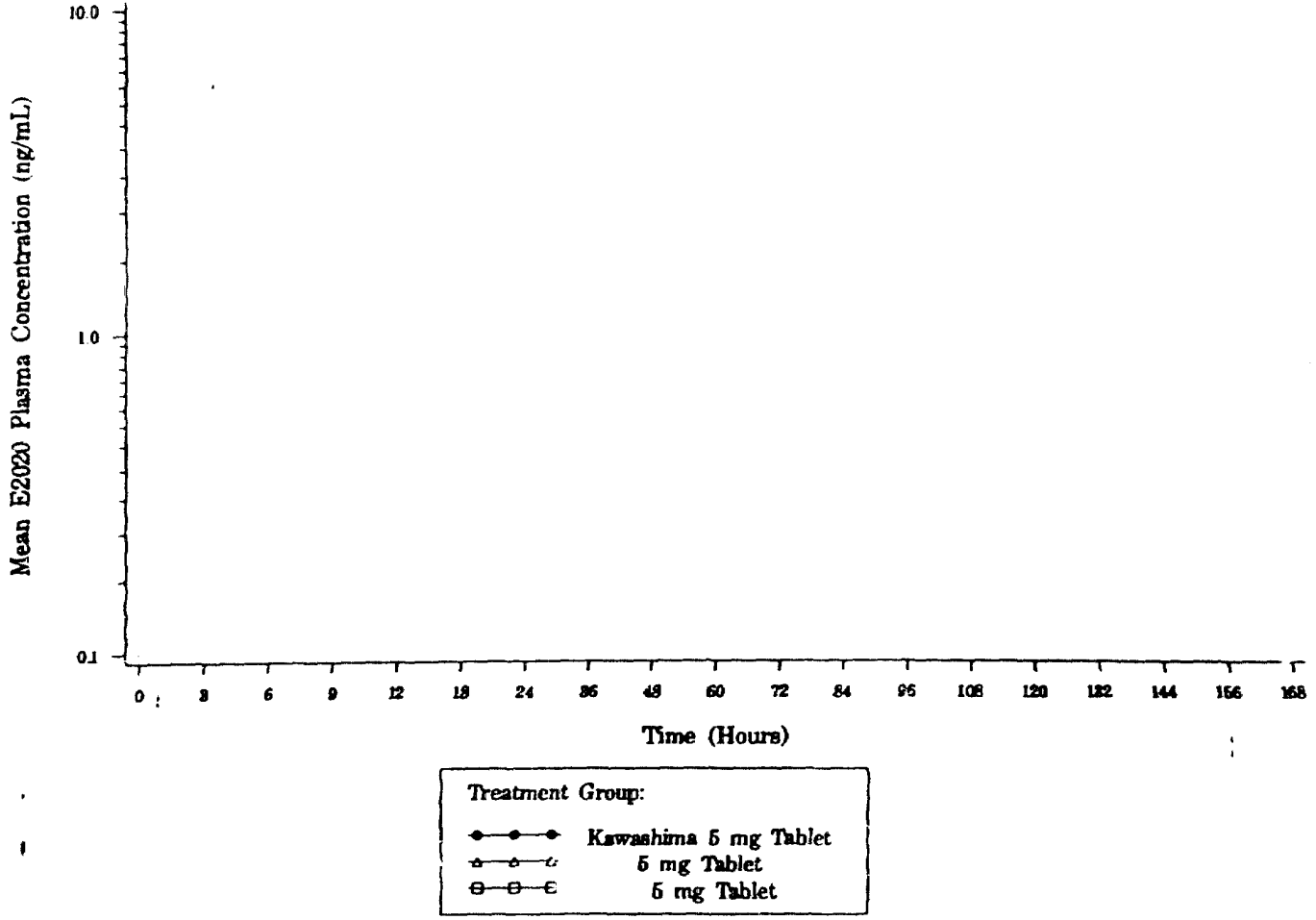
R = Kawashima is the reference formulation.

* 90% confidence interval on the ratio of test formulation to the reference formulation.

@ Schuirmann's Two one-sided tests:
Formulations are deemed bioequivalent if the classical (shortest) 90% Confidence Interval is within the 80-120% interval for the raw data, and within the 80-125% interval for the log-transformed data.
Bioequivalance is also declared if the absolute value of both t-tests are greater than 1.694

Figure II
E2020 – A001 – 009

E2020 Plasma Concentration (ng/mL)



BIOEQUIVALECE STUDY (AVSD)

CLINICAL STUDY SYNOPSIS

Title: A Study of the Bioequivalence of Two Tablet Formulations of E2020, in Healthy Volunteers; Study #: E2020-A001-010

Investigator:

Objective: The objective of this study was to determine the bioequivalence of a new 5-mg E2020 tablet formulation relative to the E2020 reference 5-mg tablet formulation used in all clinical trials.

Study Design: This study was a randomized, single-dose, open label, two-period crossover study which was conducted at a single investigational site. Each treatment period was followed by a 2-week washout period.

Study Population: Participants in this study were healthy, ambulatory, non-smoking male volunteers, aged 18-45 years. A total of 12 volunteers were randomized into this study and all 12 completed the study without incident. All participants were Caucasion, and ranged in age from 19 to 44 years and in weight from 68.5 to 86.0 kg.

Treatment Groups: Subjects randomly received each of the two following E2020 formulations:

Formulation A: 5.0-mg, film-coated E2020 tablets produced by Eisai Company, Ltd. in Kawashima, Japan. This is the REFERENCE formulation. Lot # K46016ZZZ

Formulation D: 5.0 mg, film-coated tablets produced by Pfizer Inc., Brooklyn, New York, USA. This is the TEST formulation. Lot # N5111

Medication was administered after a fast of at least eight hours and was accompanied by a total of 250-ml of tap water.

Methods *(Clinical)*: Volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to the start of the study. For each Treatment period, subjects were admitted to the study site on the evening of day 0, at least 12 hours prior to drug administration. Upon admission, a routine physical examination was conducted. Entry vital signs (sitting position), and weight were recorded, and urine specimens for drug use/abuse screening were collected. A light snack was served 10 hours prior to drug administration, after which an absolute fast, from both food and fluids (except water), was maintained. The following morning, prior to drug administration, clinical laboratory and analytical samples were collected. During each

treatment period subjects received a single 5-mg tablet of E2020. The order of formulation administration was randomized. Following drug administration, blood samples for analytical determinations were collected at specified intervals for 24 hours. Subjects were then discharged from the study site the following morning, after providing a 24-hour blood sample. Volunteers returned to the study site at 24-hour intervals (to 168 hours), for additional analytical sample collection. Total in-house time for each treatment period was approximately 36 hours. Each treatment period (inpatient + outpatient) was 7 days in duration, and was followed by a 2-week washout period.

Methods *(Analytical)*: E2020 concentrations in plasma were determined by [redacted]. The limits of quantitation for the E2020 pharmacokinetics were characterized using the resultant analytical data and standard techniques. Parameters used for between-treatment comparisons included C_{max}, Log_{10} C_{max}, $AUC_{(0-168)}$, Log_{10} $AUC_{(0-168)}$, $AUC_{(0-\infty)}$, t_{max} and $t_{1/2}$.

Results: Pharmacokinetics: For the primary pharmacokinetic parameters $AUC_{(0-168)}$ and C_{max}, the test formulation was found to be bioequivalent to the reference formulation when using both the original data scale, as well as the log-transformed data. Similar results were observed for both t_{max} and $t_{1/2}$. All of the 90% Confidence Intervals for the ratio of test formulation/reference formulation were well within the 80% - 120% interval for the raw data, and within the 80% - 125% interval for the log transformed data.

The primary pharmacokinetic parameters and 90% Confidence Intervals for the ratio of test/reference are shown in the table below.

Comparison of Pharmacokinetic Parameters (Mean ± SE)

	C_{max} (ng/ml)	Log_{10} C_{max} (ng/ml)	$AUC_{(0-168)}$ (ng*hr/ml)	Log_{10} $AUC_{(0-168)}$ (ng*hr/ml)
Formulation A (Kawashima - Ref.)	7.4 (0.2)	0.837 (.019)	358.2 (30.0)	2.535 (.041)
Formulation D (Pfizer - Test)	7.5 (0.3)	0.829 (.019)	349.0 (31.1)	2.521 (.043)
Ratio: Test / Reference	98.5%	99.04%	97.4%	99.4%
90% C. I. for ratio: Test/Reference	93.8 - 102.0	94.0 - 102.1	94.8 - 100.0	93.9 - 100.0

Individual bioequivalence for subjects receiving the test formulation was observed in 11/12 subjects (92%) for $AUC_{(0-168)}$, and 12/12 subjects (100%) for C_{max}.

Safety: No serious or unexpected adverse experiences occurred during the course of this study. Treatment Emergent Signs and Symptoms (TESS) were transient and all were mild or moderate in severity.

No clinically significant, treatment emergent abnormal laboratory values (TEAV's) were observed during the course of this study.

Conclusion:

The conclusion from this study is:

- The test formulation, Formulation D (Pfizer), is bioequivalent to the reference formulation, Formulation A (Kawashima).

For the test formulation, bioequivalence relative to the reference formulation was clearly demonstrated in both mean bioavailability, as well as in bioavailability in individual subjects.

Eisai America, Inc.
Protocol: E2020-A001-010
Investigator:

Table 8
A Comparison of Pharmacokinetic Parameters

			PFIZER vs. KAWASHIMA[R]	
	PFIZER E2020 5mg	KAWASHIMA[R] E2020 5mg	90% C.I. for Ratio* Test/Reference	Two One-sided@ t-tests (T_U, T_L)
$AUC_{0\text{-}168\ hrs.}$ (ng*hr/mL)				
N	12	12		
Mean (S.E.)	349.0 (31.1)	358.2 (30.0)	(94.8 , 100.0)	(-15.13 , 11.66)
Min - Max	195.3 - 495.3	188.5 - 499.5		
Coeff. Var.	5.3%	5.2%		
$log_{10}AUC_{0\text{-}168\ hrs.}$ (ng*hr/mL)				
N	12	12		
Mean (S.E.)	2.521 (.043)	2.535 (.041)	(93.9 , 100.0)	(-13.91 , 10.46)
Min - Max	2.291 - 2.695	2.275 - 2.699		
Coeff. Var.	0.042%	0.042%		
Geometric Mean	331.9	342.6		
$AUC_{0-\infty}$ (ng*hr/mL)				
N	12	12		
Mean (S.E.)	487.1 (34.5)	480.9 (36.7)	(99.0 , 103.6)	(-13.93 , 15.86)
Min - Max	314.8 - 711.6	259.9 - 707.9		
Coeff. Var.	4.6%	4.7%		

Data Source: Statistical Appendix VII.

R = Kawashima is the reference formulation.

** 90% confidence interval on the ratio of test formulation to the reference formulation.*

@ Schuirmann's Two one-sided tests:

Formulations are deemed bioequivalent if the classical (shortest) 90% Confidence Interval is within the 80-120% interval for the raw data, and within the 80-125% interval for the log-transformed data.

Bioequivalance is also declared if the absolute value of both t-tests are greater then 1.717

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Eisai America, Inc.
Protocol: E2020-A001-010
Investigator:

Table 8
A Comparison of Pharmacokinetic Parameters

	PFIZER E2020 5mg	KAWASHIMA® E2020 5mg	PFIZER vs. KAWASHIMA®: 90% C.I. for Ratio* Test/Reference	PFIZER vs. KAWASHIMA®: Two One-sided@ t-tests (T_U, T_L)
C_{max} (ng/mL)				
N	12	12		
Mean (S.E.)	6.8 (0.3)	6.9 (0.3)	(93.8 , 102.0)	(-9.24 , 7.50)
Min - Max	5.0 - 8.0	5.0 - 8.8		
Coeff. Var.	8.5%	8.3%		
$Log_{10}C_{max}$ (ng/mL)				
N	12	12		
Mean (S.E.)	0.829 (.019)	0.837 (.019)	(94.0 , 102.1)	(-10.10 , 8.43)
Min - Max	0.698 - 0.902	0.699 - 0.944		
Coeff. Var.	0.055%	0.055%		
Geometric Mean	6.7	6.9		
t_{max} (Hour)				
N	12	12		
Mean (S.E.)	3.9 (0.3)	4.1 (0.3)	(83.1 , 108.7)	(-3.22 , 2.13)
Min - Max	2.0 - 6.0	2.0 - 6.0		
Coeff. Var.	27.0%	25.9%		
$t_{1/2}$ (Hour)				
N	12	12		
Mean (S.E.)	77.72 (3.56)	70.86 (4.20)	(103.9 , 115.5)	(-3.05 , 8.78)
Min - Max	57.89 - 97.60	37.77 - 90.85		
Coeff. Var.	10.7%	11.7%		

Data Source: Statistical Appendix VII.
R = Kawashima is the reference formulation.
* 90% confidence interval on the ratio of test formulation to the reference formulation.
@ Schuirmann's Two one-sided tests:
Formulations are deemed bioequivalent if the classical (shortest) 90% Confidence Interval is within the 80-120% interval for the raw data, and within the 80-125% interval for the log-transformed data.
Bioequivalance is also declared if the absolute value of both t tests are greater then 1.694

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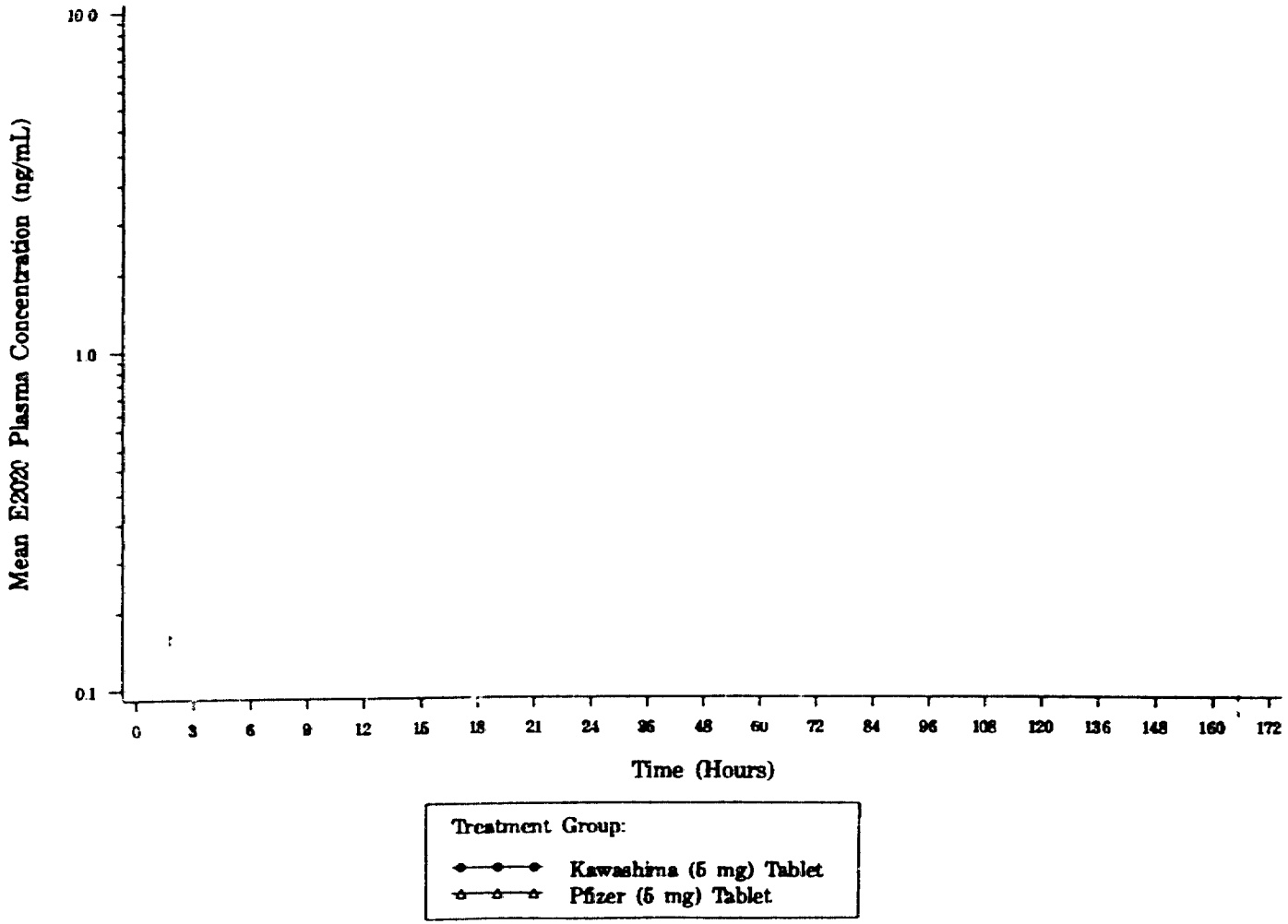
Figure II
E2020 – A001 – 010

E2020 Plasma Concentration (ng/mL)



Food EFFECT

CLINICAL STUDY SUMMARY

Title: A single oral-dose crossover study to evaluate the effect of food on the pharmacokinetics of E2020 in healthy male subjects.

Principal Investigator:

S

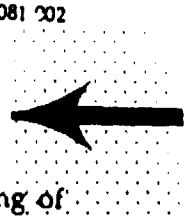
Objective: To evaluate the effect of food on the pharmacokinetic profile of E2020 after single, oral administration of 2 mg of E2020 to healthy, male subjects.

Study Design: This single, oral-dose, phase I study was designed to examine the effect of food on the pharmacokinetic profile of E2020 in healthy male subjects. A total of 12 healthy, male subjects received a single dose of E2020 in both fasted and non-fasted conditions according to a crossover design. Subjects were enrolled in this study and were randomized into two groups of 6 each. Group 1 received a single 2-mg dose of E2020 in a fasted condition during Period 1 and after a standard breakfast during Period 2. Group 2 received drug after a standard breakfast during Period 1 and received the drug in a fasted condition during Period 2. A 32-day wash-out period separated the two treatment periods.

Methods: This single, oral-dose, 2-period crossover study was performed in order to evaluate the effect of food on the safety and pharmacokinetics of E2020. Safety and pharmacokinetic evaluations were made during the 12-day period following drug administration.

The safety evaluation of E2020 was based upon the assessment of the results of the following: physical examinations, recording of subjective symptoms, electrocardiogram recordings, vital signs, ophthalmological examinations, and clinical laboratory tests.

Concentrations of E2020 in plasma were determined by a sensitive, specific procedure. Model-independent pharmacokinetic parameters were determined by standard methods.



4.3.7.2 Drug Administration

In each leg of the crossover, 12 subjects received a single, oral dose of 2 mg of E2020 with 120 ml of water. Six (6) subjects received it after fasting for 12 hours and 6 received it within 5 minutes after breakfast, which was served between 08:40 and 08:55.

4.3.7.3 Breakfast for Non-fasting Subjects

The breakfast served to the non-fasting subjects prior to E2020 administration included 2 pieces of roll, jam (15 g), scrambled eggs, sauteed ham, lettuce, cucumber, tomato, and beef consomme soup. They were required to consume all the food they were served.

4.3.7.4 Procedures

During the Treatment Phase, the following assessments were performed:

- Physical examinations

  Physical examinations were carried out 1, 2, 4, 8, 24 (Day 2), and 48 (Day 3) hours after dosing.

- Signs and symptoms

  Subjective symptoms were recorded, using questionnaires, at 0.5, 1, 2, 3, 4, 6, 8, 24 (Day 2) and 48 (Day 3) hours after dosing.

- Ophthalmological examinations

  Funduscopy* using a

  measurements of visual acuity using an International sight-testing chart read at 3m from the subject, ocular tension by air puff method using a

  and pupil size measurement by Distantometer) were conducted at 4, 24 (Day 2) and 48 (Day 3) hours after dosing.

* Fundus photography was conducted by a technician and the photograph was evaluated by an ophthalmologist.

Study Population: A total of 12 healthy, male Japanese subjects, aged 20 to 27, were enrolled into this study.

Results: Safety

E2020 was generally well tolerated by all subjects. However, some complaints and objective findings were temporally related to E2020 dosing and a relationship with the study drug could not be excluded. Symptoms that were considered possibly-related to E2020 included mild sleepiness in one subject on two occasions and mild headache in two subjects. These symptoms were brief and self-limited in each instance. The only objective finding that was considered possibly-related to E2020 dosing was the presence of dry rales in the right lung of one subject noted at follow-up. The remainder of the symptoms reported by the subjects, and objective findings on examination, were judged by the investigator to be unrelated to E2020.

One subject had increases in standing heart rate following his fasting-dose of E2020 up to a maximum of 132 beats per minute. His electrocardiograms all showed sinus rhythm and his supine pulse rates were almost all less than 100 beats per minute. His pulse rate did not increase following his non-fasting dose. It is unlikely that this increase in pulse rate is related to E2020, since cholinesterase inhibitors such as E2020 should lower heart rate. No other clinically significant changes in vital signs were observed in this study.

A number of minor clinical laboratory value abnormalities were noted during the study. All were considered clinically insignificant.

Pharmacokinetics:

The following model-independent pharmacokinetic parameters* are listed in the table below: maximum concentration (C_{max}), time of C_{max} (T_{max}), area under the plasma concentration-time curve from the time of dosing to 168 hours (AUC_{0-168}).

* Pharmacokinetic parameters are for the free base of E2020 while the dose is quantitated as E2020 itself (the hydrochloride salt).

Table 16-3 Mean (±SD) Model Independent Pharmacokinetic Parameters for E2020

	C_{max} (ng/ml)	(t_{max}) (hr)	AUC_{0-168} (ng × hr/ml)	$t_{1/2}$ (hr)	$AUC_{0-\infty}$ (ng × hr/ml)
Fasting	3.31 ± 0.49	3.00 ± 1.21	166.5 ± 46.8	66.9 ± 20.7	213.6 ± 65.0
Non-Fasting	3.22 ± 0.29	3.42 ± 1.51	172.8 ± 32.5	91.6 ± 33.2	247.4 ± 57.0

* ($P<0.05$, Analysis of Variance)

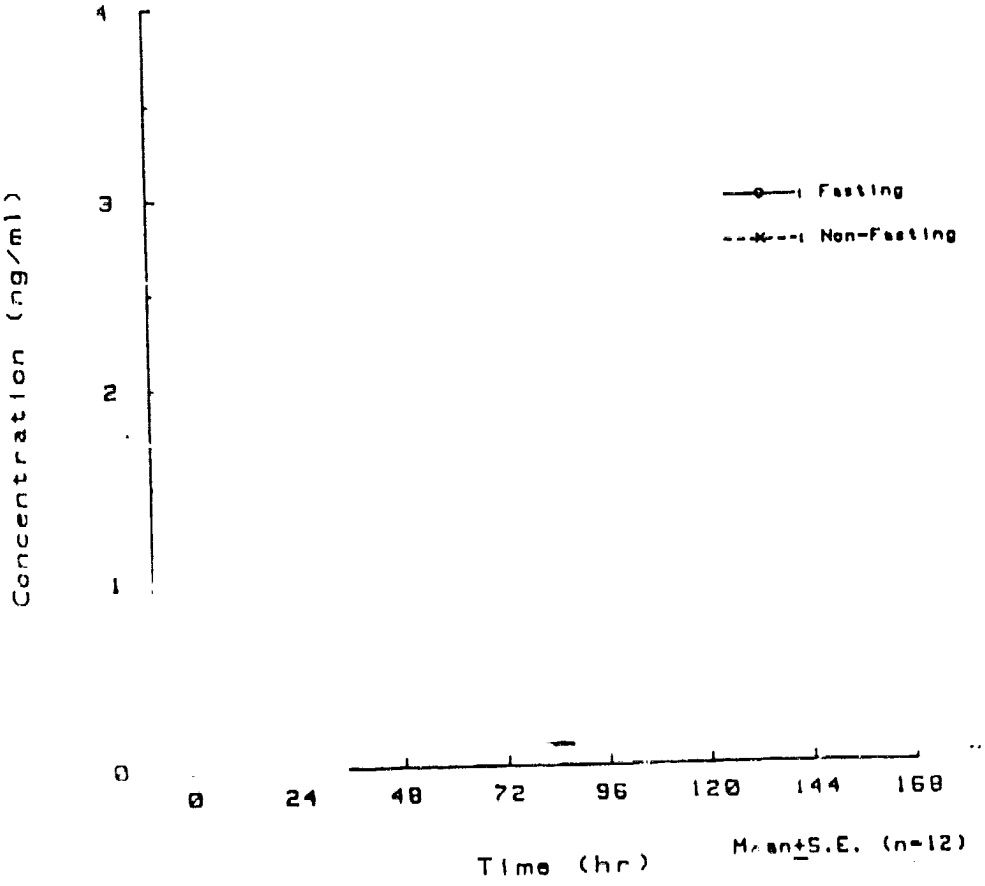


Fig. 2 Mean Plasma Levels of E2020

DOSE PROPORTIONALITY AT MULT DOSE (1,3,&5mg)

CLINICAL STUDY SYNOPSIS

Title:	An Ascending, Multiple-Dose, Safety and Tolerance Study of E2020, a Cholinesterase Inhibitor, in Healthy Male Volunteers; Study E2020-A001-002
Investigators:	
Objectives:	The primary objective of the study was to assess the safety and tolerance of E2020 in healthy men, following administration of the drug for 21 consecutive days as single, oral doses. Ascending dose strengths were evaluated in sequential study periods. The secondary objectives, at each dose level, were the measurement of E2020 concentrations in plasma and the determination of cholinesterase activity in plasma and red blood cells.
Study Design:	This study was a single-center, double-blind, randomized, placebo-controlled, sequential-group, multiple-dose study. Three dose levels were investigated: 1.0 mg, 3.0 mg, and 5.0 mg. At each dose level a sufficient number of volunteers were enrolled to allow eight subjects to complete each evaluation period. Two subjects of each group were randomized to receive placebo; the remaining six were randomized to receive E2020. Progression to the next higher dose level was contingent upon demonstration of tolerance of E2020 at the previous dose level. The subjects were sequestered a for at least 30 days and 31 nights.
Drug Administration:	Three tablets of study medication (E2020 or placebo) were administered after a fast of at least twelve hours and were accompanied by 250 ml of tap water for 21 consecutive days.
Methods:	After a fast of at least twelve hours, the subjects received 21 consecutive, daily doses of E2020 or placebo. Safety and tolerance were monitored for 30 days (720 hours) following the initial drug administration. The safety evaluation of E2020 was based upon the results of the following: physical examinations, reported and observed adverse events (AEs),

vital signs, electrocardiogram recordings, and clinical laboratory tests.

Concentrations of E2020 in plasma were determined by a sensitive and specific procedure. Cholinesterase activity in red blood cells (rbc ChE) was assessed by a specific radioenzyme procedure. Blood samples were collected at specified time intervals during the 30 days (720 hours) following the initial drug administration. Matrices for each analyte were taken from the same collection tube.

Study Population: A total of 27 healthy, male subjects were enrolled into this study. Three subjects were removed from the study because of baseline abnormal laboratory values. Among the participants, 24 were white and three were black. Participants ranged in age from 19 to 40 years and ranged in weight from 65.0 to 85.0 kg.

Results:

Safety: Most of the adverse events reported in the study were transient and mild or moderate in severity. Some were temporally related to E2020 administration, and, in these cases, a relationship with the study drug could not be completely excluded. The proportions of subjects reporting adverse events in the E2020 dose-groups were similar to the corresponding proportions in the placebo groups. There was no apparent relationship between the occurrence of adverse events and dose level of E2020.

Minor clinical laboratory value abnormalities were noted during the study. Most were clinically insignificant and none were considered likely to be related to E2020 administration.

Pharmacokinetics: The pharmacokinetics of E2020 appeared to be linear, and stationary over the course of the study. A difference was observed for the λ_z values of the 1.0-mg group; this difference may be an artifact or may reflect a true difference in the disposition of E2020 at the lowest dose. Accumulation of E2020 occurred over the 3-week period of daily dosing; predicted and observed accumulation ratios were generally in good agreement.

Pharmacodynamics: A significant relationship between E2020 plasma concentration and inhibition of red blood cell

cholinesterase was established and no time-dependent or hysteresis in pharmacologic response was observed.

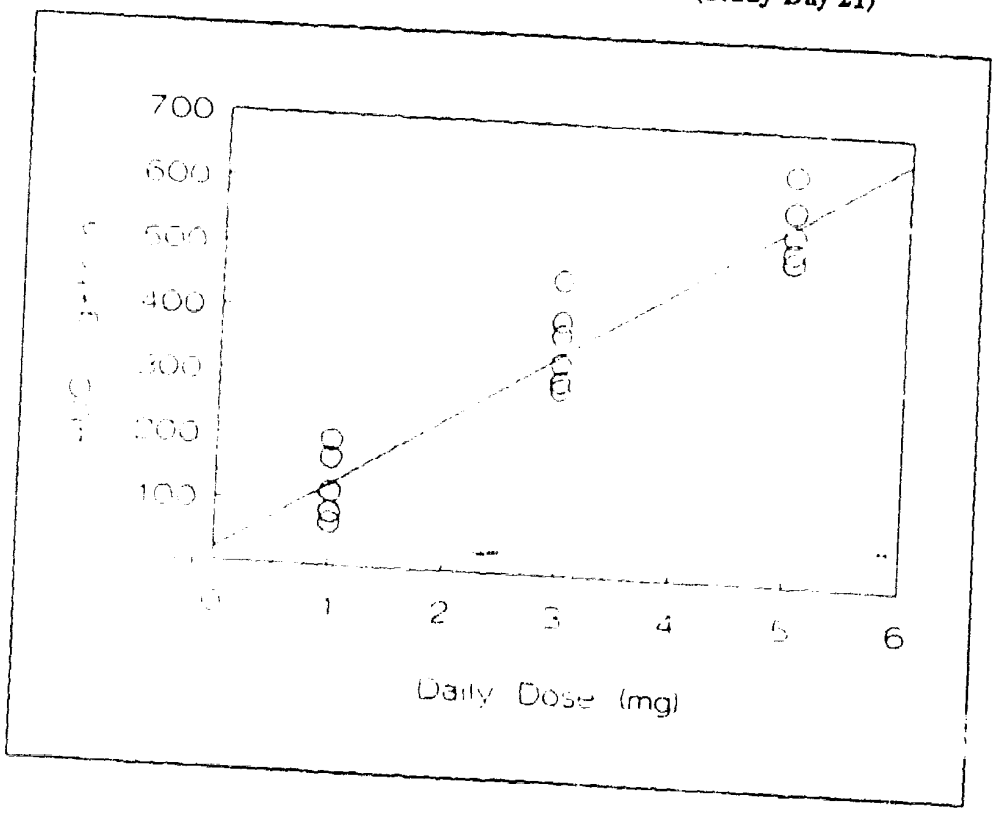
Reliable prediction of steady-state effect may be made on the basis of E2020 plasma concentrations or inhibition of red blood cell cholinesterase.

Conclusions:

The conclusions that resulted from this trial are:

- E2020 is generally well tolerated by normal subjects and is safe at the doses and duration of administration used in this study.
- The pharmacokinetics of E2020 appeared to be linear and stationary over the course of the study; steady state of E2020 concentration is achieved after administration for approximately three weeks.
- The inhibition of cholinesterase associated with the administered of E2020 can be characterized by simple models.

Figure VI **The relation between dose and Area Under the Curve for E2020 during multiple dose administration (Study Day 21)**



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Y = 107X + 19.9, r^2 = 0.916, $p < 0.0001$

Figure IX The relation between Dose-Normalized Area Under the Curve and Number of Days of E2020 administration

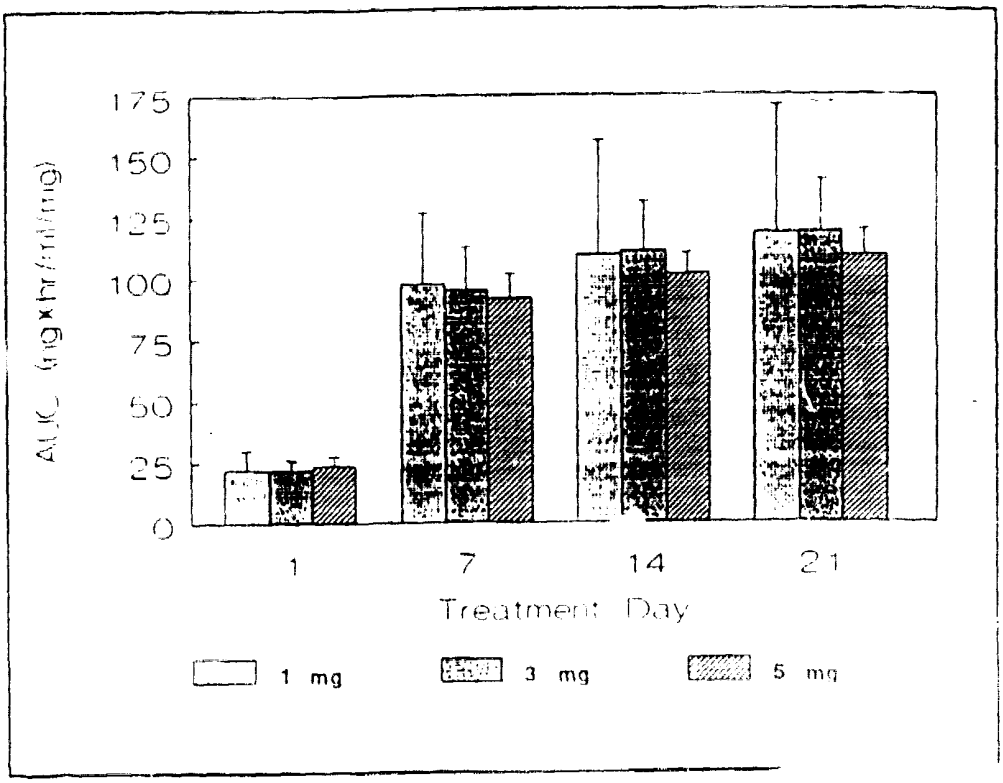


Table XX Model-independent pharmacokinetic parameters for E2020 during 21 days of once-daily administration (Mean ± sd)

Dose (mg)	$AUC\|_{0-t}$ (ng•hr/ml) Day 1	Day 7	Day 14	Day 21	λ_z (hr^{-1})	$t_{1/2}$* (hr)	Cl/F (L/hr/Kg)	V_{ss}/F (L/Kg)
1.0	22.2± 7.8	97.9± 29.2	109.8± 46.7	118.5± 52.3	0.00706± 0.00214	98.2± 29.8	0.132± 0.060	18.1± 3.8
3.0	66.3± 11.9	286.8± 51.9	334.0± 60.2	357.7± 64.0	0.00951± 0.00132	72.9± 10.1	0.120± .020	12.8± 2.5
5.0	118.0± 18.6	460.3± 49.6	509.1± 43.2	546.5± 52.6	0.01031± 0.00152	67.3± 9.9	0.125± 0.016	12.3± 1.9

Table XXI E2020 accumulation during once daily administration for 21 days (mean ± sd)

Dose (mg)	Accumulation ratio Predicted[a]	Observed[b]	Days to steady-state[c]
1.0	6.92± 2.03	6.16± 4.02	22.20± 7.04
3.0	4.97± 0.57	5.43± 0.61	15.41± 2.00
5.0	4.65± 0.69	4.72± 0.78	14.30± 2.40

[a] Calculated as 1/(1-e^-λz)

Figure VII Terminal disposition constant calculated from log linear phase

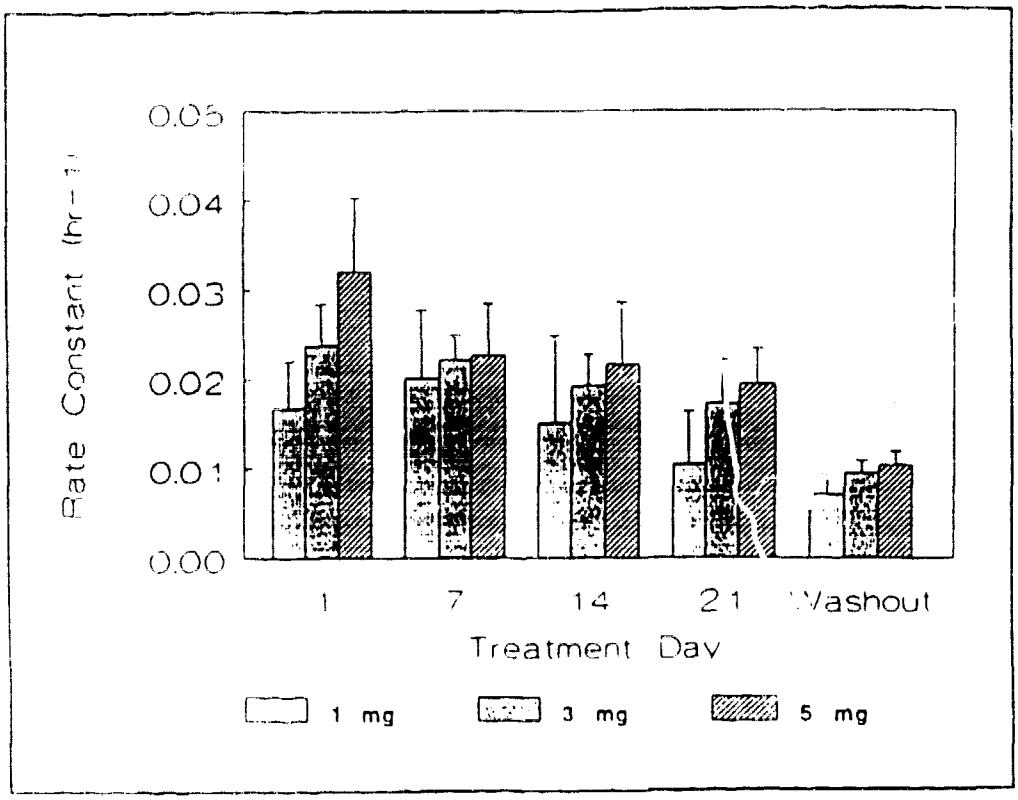


Figure VIII The relation between Area Under the Curve and Number of Days of E2020 administration

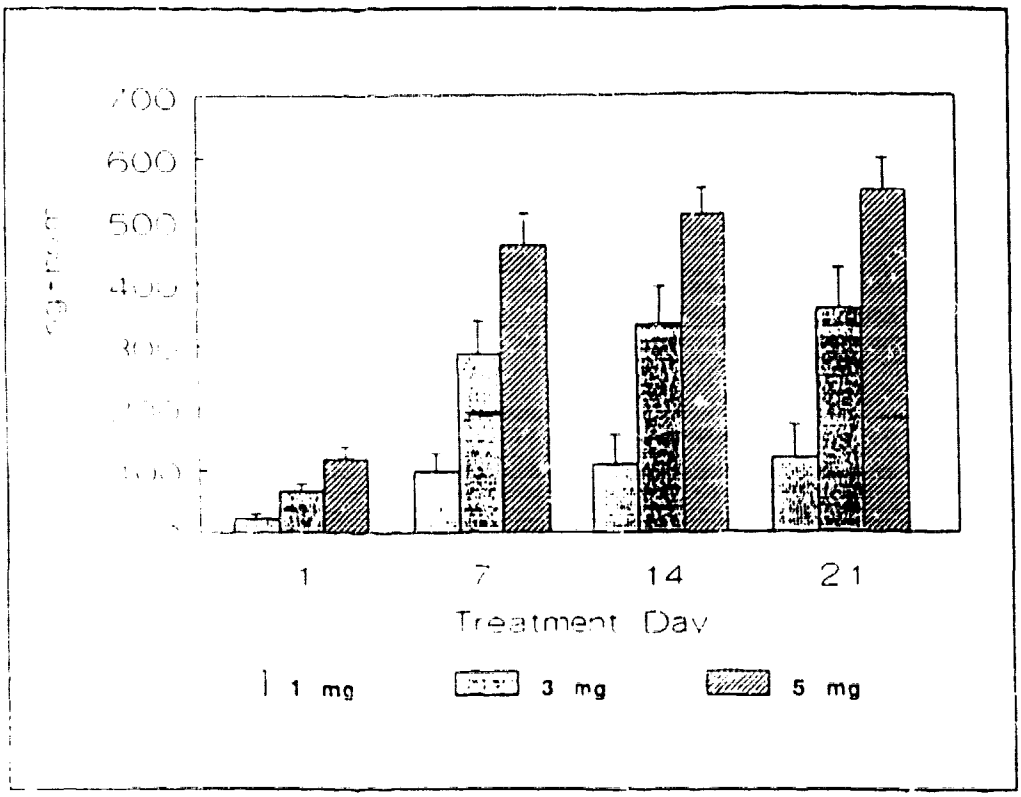


Figure XIV The Effect of Duration of Treatment on E2020 AUE (mean ± SE)

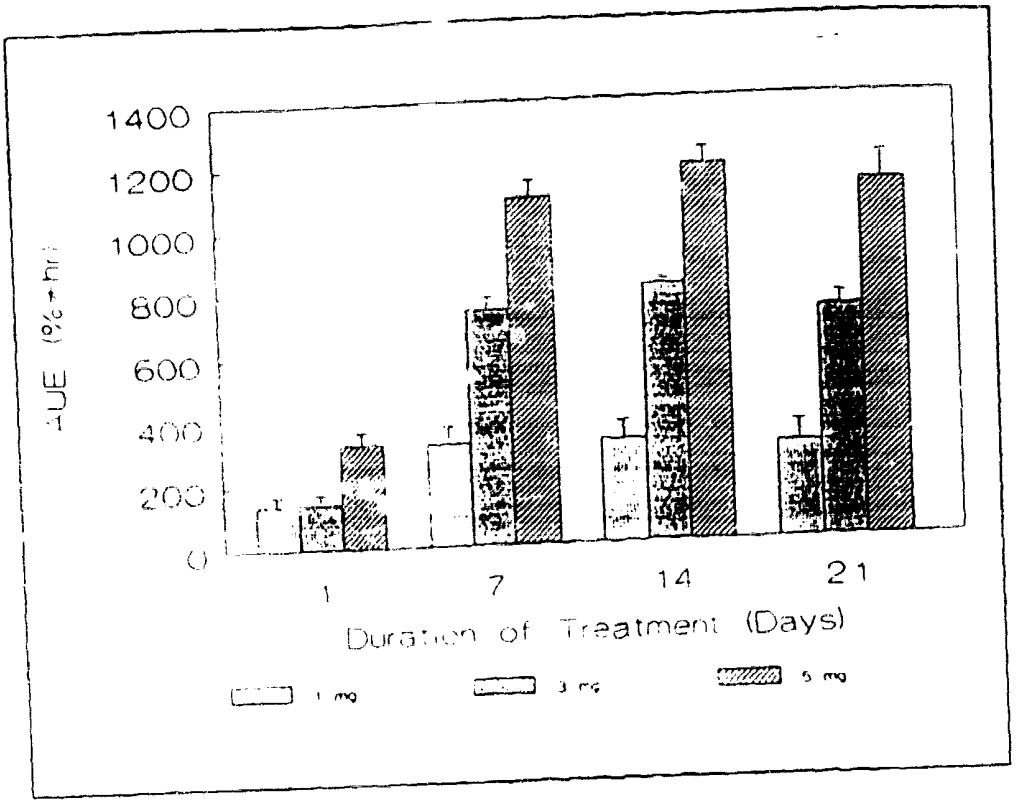
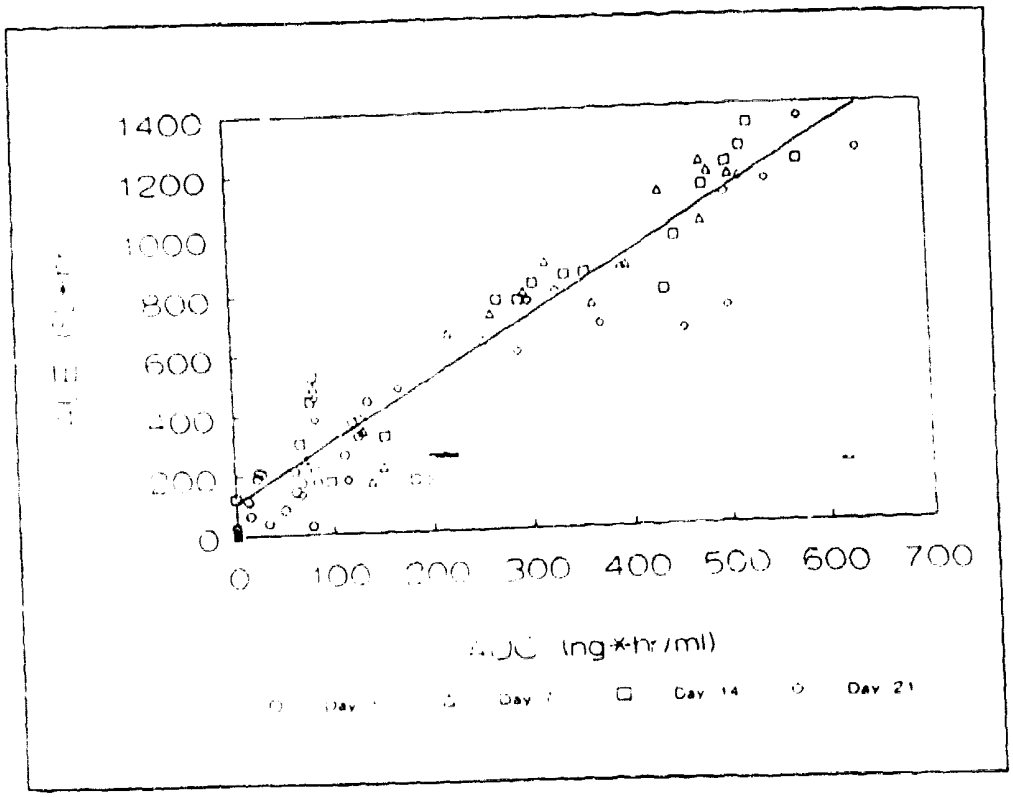


Figure XV The Relation between AUE and AUC during multiple administration of E2020



parameters, approximately 90% of the interindividual differences in rChEI by E2020 can be ascribed to intersubject variability in E2020 disposition.

The results of fitting both linear and simple E_{max} pharmacodynamic models to the individual E2020 effect versus concentration data are compiled in Table XXII.

Table XXII Pharmacodynamics of E2020 during 21 days of administration

Dose (mg)	Subject #	Linear Effect Model			E_{max} Effect Model			Optimal Model
		Slope (%/ng/ml)	Intercept (%)	AIC*	E_{max}(%)	EC_{50} (ng/ml)	AIC	
1.0	2							
	3							
	6							
	7							
	28							
	29							
3.0	9							
	10							
	12							
	13							
	16							
	39							
5.0	18							
	19							
	20							
	21							
	22							
	24							
	Mean							
	SD							

*Akaike's Information Criterion

The linear effect model was statistically superior to the E_{max} model for 9 of the 18 data sets. The subjects for which the linear model was superior tended to receive lower doses; the E_{max} model was statistically superior for all six subjects from the 5.0-mg-dose group and for three subjects from the 3.0-mg-dose group. On examination of the behavior of each model across all subjects, the linear effect model predicted that, on average, rChEI would increase by approximately 2.3% per unit (ng/ml) increase in C_p of E2020. As would be expected, the mean intercept across experimental subjects (3.53 ± 3.45%) was not statistically different from 0. When considering only those subjects for whom the linear

Figure XVI The Relation between Cholinesterase Inhibition and E2020 Concentration During Multiple Dose Administration, Linear Effect Model

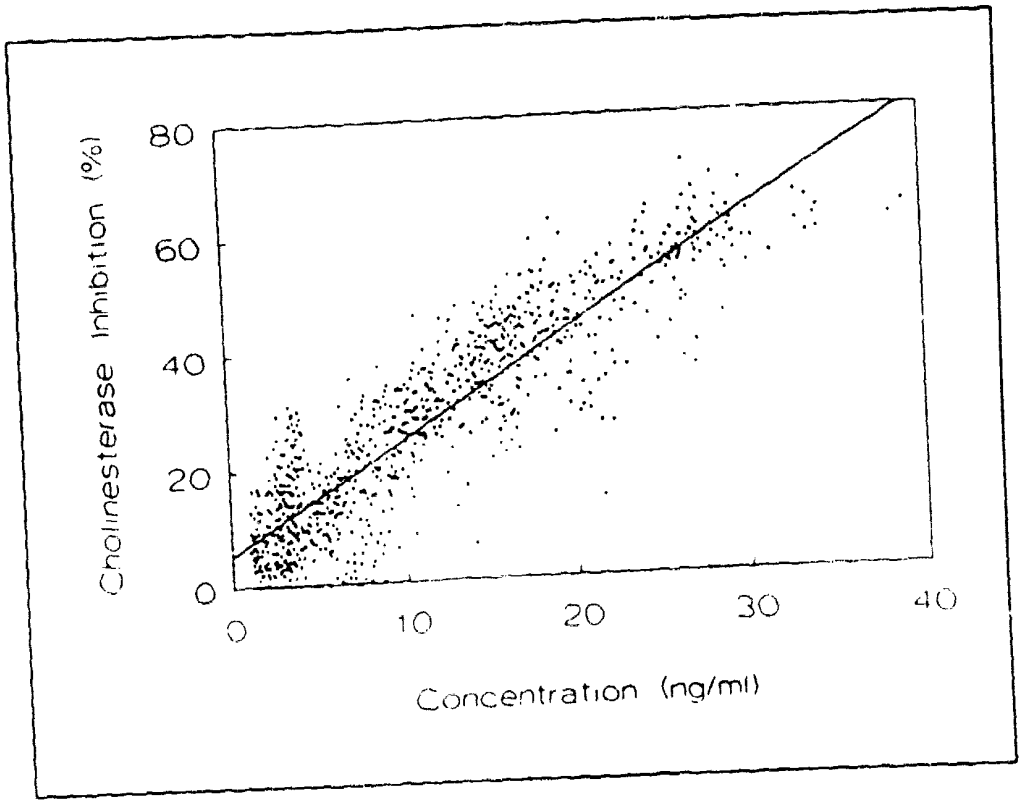
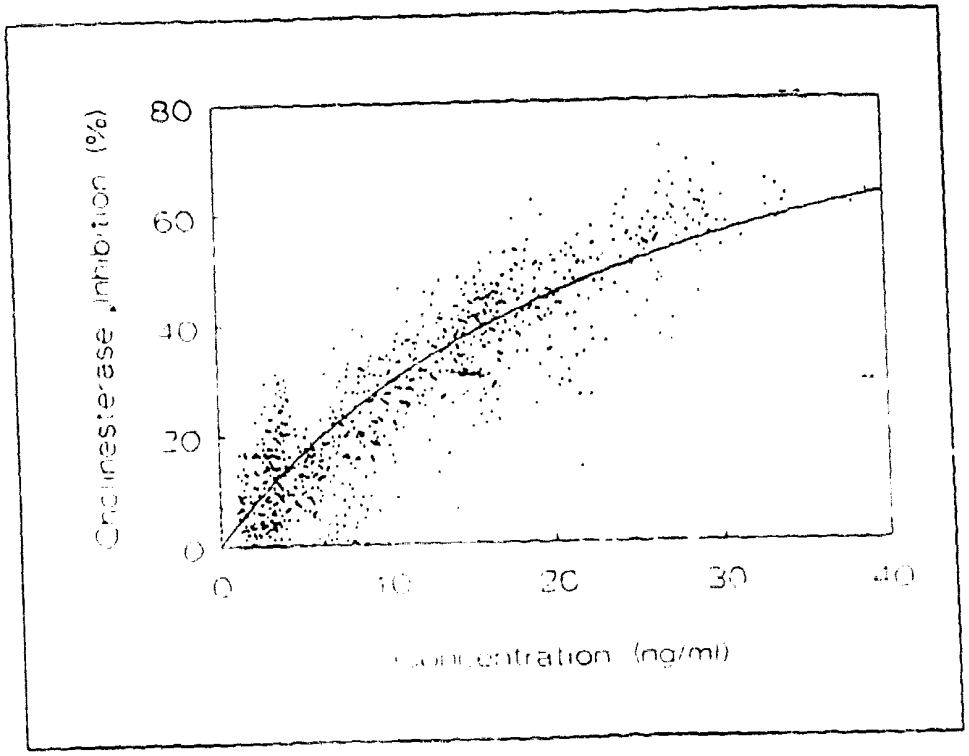


Figure XVII The Relation between Cholinesterase Inhibition and E2020 Concentration During Multiple Dose Administration, Simple E_{max} Model



STEADY STATE KINETICS, PK/PD, & DP (5 & 10mg)

CLINICAL STUDY SYNOPSIS

Title: A Multiple-Dose Study of the Pharmacokinetics and Peripheral Pharmacodynamics of E2020, During Evening Administration in Healthy Volunteers. Study #: E2020-A001-011

Investigator:

Objective: The primary objective of this study was to establish a pharmacokinetic (PK) profile of E2020 during repeated evening administration of either 5-mg or 10-mg single daily doses for a total of 28 days. The secondary objective was to measure the peripheral pharmacodynamics of E2020 associated with the PK profile. A tertiary objective was the measurement of E2020 plasma protein binding of at steady state.

Study Design: This study was a double-blind, randomized, multiple-dose study of 2 dose levels of E2020.

Study Population: Volunteers for this study were healthy, ambulatory, non-smoking male and female volunteers, greater than 18 years of age. A total of 16 volunteers were randomized and 14 volunteers completed this study. All participants were Caucasian. The 5-mg group was comprised of 7 males and 1 female and ranged in age from 19 to 56 years and in weight from 60.4 to 78.3 kg. The 10-mg group was comprised of 6 males and 2 females and ranged in age from 20 to 47 years and in weight from 51.4 to 93.0 kg. Demographic characteristics are summarized in the table below.

Demographics Summary

	Male	Female	Age (yrs) Mean (± S.E.)	Weight (kg) Mean (± S.E.)
5-mg dose	7	1	40.1 (4.0)	70.8 (2.2)
10-mg dose	6	2	36.1 (3.4)	80.2 (6.1)

Treatment Groups: Study subjects were randomized to receive either 5.0 or 10.0 mg daily doses of E2020 for 28 days. To minimize reactions to acute, extensive inhibition of acetylcholinesterase, the 10-mg dose was administered using a scheduled-titration scheme. Subjects randomized to the 10-mg group initially received 5-mg daily doses of E2020 for 7 days before receiving their first 10-mg dose. The 10-mg daily dose was then maintained for the remaining 21 days of the study.

Methods (*Clinical*): After providing written informed consent, volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to randomization into the study. Appropriate subjects were admitted to the study site in the morning, at least 12 hours prior to drug administration. Following admission, body weight was recorded and baseline physical examinations and urine drug screens were conducted. Four hours prior to drug administration (18:00), a standard evening meal was provided, after which an absolute fast from food and fluids (except water) was maintained until the following morning. Beginning that evening at 22:00, subjects received their first single daily oral dose of E2020 with 250 ml of tap water. Following drug administration, vital signs were recorded regularly and blood samples were collected at specified intervals. Monitoring for adverse effects was conducted continuously.

Drug administration continued at 22:00 hours each evening for 27 additional days. Subjects were inpatients for 37 days in order to allow for characterization of the terminal disposition phase of E2020. Blood samples were collected at specified intervals for the measurement of E2020 in plasma, acetylcholinesterase activity in red blood cells and plasma protein binding of E2020. Laboratory evaluations were conducted on days 1, 7, 14, 21, 28 and 37. On Days 14, 28 and 37, physical examinations were repeated. Subjects were discharged on the evening of study day 37.

Methods (*Analytical*): Blood samples for the measurement of E2020 plasma concentrations and red blood cell acetylcholinesterase inhibition (rbc-AChEI) were collected just prior to each dose (C_{min}) during the 28 days of drug administration and at specified times during 24-hour periods on Days 1, 7, 14, 21 and 28. Following the last dose on Day 28, blood samples were collected out to 216 hours. Blood samples for the determination of E2020 protein binding were taken at specified times during this 216-hour terminal disposition period.

E2020 concentrations in plasma were determined by

Inhibition of acetylcholinesterase activity in red blood cells (rbc-AChEI) was assessed using a specific radioenzyme assay. Protein binding of E2020 in plasma was measured using an equlibrium dialysis method. E2020 pharmacokinetics and pharmacodynamics were characterized using the resultant analytical data and standard techniques. The area under the plasma concentration versus time curve from 0-24 hours (AUC_{0-24}) was evaluated on Days 1, 7, 14, 21 and 28. Data collected from Day 28 to Day 37 was used to calculate the terminal disposition rate constant (λ_z) and half-life ($t_{1/2}$). Pharmacodynamic comparisons between dose groups were done using % rbc-acetylcholinesterase inhibition (% AChEI).

5.1 PHARMACOKINETICS

E2020 plasma concentrations for each individual subject are presented by blood sampling timepoint in DATA LISTING 17. Summary statistics for each timepoint and each E2020 dose are presented in TABLE 7. A Time versus Concentration curve of the mean data (± SE) for both doses is presented in FIGURE II.

A summary of steady state pharmacokinetic (PK) parameters for both doses is given in the table below. Values for Clearance (Cl_{ss}) and for Volume of distribution (V_z) were adjusted for body weight.

All calculations for the PK parameters listed can be found in APPENDIX VII (Statistical Output). A summary of the PK parameters calculated for the 24-hour sampling periods on days 1, 7, 14, 21 and 28 can be found in TABLE 12.

E2020 Pharmacokinetic Parameters at Steady State (Mean ± S.D.)

	C_{max} (ng/ml)	t_{max} (hrs)	$AUC_{(0-24)}$ (ng*hr/ml)	$AUC_{(0-\infty)}$ (ng*hr/ml)
5-mg dose	34.1 (7.3)	3.0 (1.4)	634.8 (92.2)	2889.3 (751.6)
10-mg dose	60.5 (10.0)	3.9 (1.0)	1127.8 (195.9)	5051.9 (1613.6)

	Cl_{ss}/F (L/hr/kg)	V_z/F (L/kg)	R_A	$t_{1/2}$ (hrs)
5-mg dose	0.11 (0.02)	11.8 (1.7)	7.18 (2.37)	72.7 (10.6)
10-mg dose	0.11 (0.02)	11.6 (1.9)	6.16 (1.06)	73.5 (11.8)

The pharmacokinetics of E2020 were observed to be linear, dose-related and stationary over the course of the study (see Figures II, III and IV). The large apparent volume of distribution (V_z) (11-12 L/kg) suggests that E2020 distributes extensively into tissue. The $t_{1/2}$ was determined to be between 72-74 hours and accumulation of E2020 was observed over a 3-week period of daily dosing. Steady-state was definitively achieved within 14 - 21 days (TABLE 10, FIGURE V). The accumulation ratio (R_A) was computed by taking the ratio of $AUC_{(0-24)}$ - Day 28 to Day 1, where the Day 1 values were multiplied by 2 for the 10-mg dose group (all subjects received 5-mg on Day 1). $AUC_{(0-24)}$ values for the 24-hour measurement periods on days 1, 7, 14, 21 and 28 are listed in TABLE 12.

The range of plasma concentrations measured for each dose at steady-state (C_{min} - C_{max}), along with the average plasma concentration at steady-state (C_{ss}), are shown in the table below. Average trough concentrations for study days 1-29 are presented in FIGURE VI.

E2020 Plasma Concentration Parameters at Steady State (Mean ± S.D.)

	C_{min} (ng/ml)	C_{max} (ng/ml)	C_{ss} (ng/ml)	$AUC_{(0-24)}$ (ng*hr/ml)
5-mg dose	21.4 (3.8)	34.1 (7.3)	26.45 (3.9)	634.8 (92.2)
10-mg dose	38.5 (8.6)	60.5 (10.0)	47.0 (8.2)	1127.8 (195.9)

5.4.1 Dose Proportionality:

Plasma concentrations of E2020 at steady-state were observed to be dose-proportional, as reflected by the C_{min}, C_{max}, C_{ss} and $AUC_{(0-24)}$ values shown in the table above. In addition, dose proportionality can also be seen in FIGURE II, the mean plasma concentration versus time profile for both dose groups over the course of the study. Statistically, dose-proportionality was demonstrated by the ratios of the least squares means for $AUC_{(0-24)}$, C_{max}, t_{max} and Clearance at steady-state for the 5 and 10-mg doses, as shown in the table below.

Dose-Proportionality - Ratio of Least Squares Means at Steady-State

	5-mg	10-mg	Ratio (%)	90% Confidence Limits
$AUC_{(0-24)}$ (ng*hr/ml)	620.4	1133.7	182.7	159.4 - 206.1
C_{max} (ng/ml)	32.5	60.6	186.1	164.9 - 207.4
t_{max} (hours)	3.2	3.5	107.8	81.2 - 134.4
Cl_{ss}/F (L/hr)	8.2	9.1	110.7	96.0 - 125.4

Because the 90% confidence intervals contained 200% for $AUC_{(0-24)}$ and C_{max} and 100% for Clearance and t_{max}, E2020 pharmacokinetics can be considered dose-proportional over the 5 to 10-mg dose range.

5.5 PHARMACODYNAMICS

A significant relationshship was observed between E2020 plasma concentration and the percent AChEI (FIGURE VII). This relationship was consistent during each of the 24-hour periods evaluated (Days 1, 7, 14, 21 and 28) and demonstrates that there are no time-dependent changes in E2020 pharmacodynamics (FIGURE VIII). Summary values at steady-state for the range of AChE inhibition (E_{min} - E_{max}) and for the average % AChE inhibition (E_{ss}), are shown in the table below for both doses. Area under the

effect curve for AChE inhibition for the 24-hour period at steady-state ($AUEC_{ss}$) is also shown. The average % inhibition (E_{ss}) was computed by integrating % inhibition versus time from 0-24 hours during the 24-hour sampling periods, and dividing by 24.

All calculations for the pharmacodynamic parameters listed can be found in APPENDIX VII (Statistical Output).

Acetylcholinesterase Inhibition Parameters at Steady State (Mean ± S.D.)

	E_{min} (%)	E_{max} (%)	E_{ss} (%)	$AUEC_{SS}$ (%*hr)
5-mg dose	62.2 (5.8)	71.8 (4.3)	65.3 (5.2)	1567.1 (124.1)
10-mg dose	74.7 (4.4)	83.6 (1.9)	77.8 (2.9)	1867.5 (71.0)

5.6 PROTEIN BINDING

The mean percent protein binding for each subject was calculated from protein binding determinations made from seven blood samples taken at specified times during the 216-hour period after the final dose on Day 28. Protein binding was consistent among all subjects and both dose groups (see APPENDIX X). A total mean value for percent protein binding was calculated using the mean percent protein binding values determined for each subject. The mean (± SD) E2020 plasma protein binding at steady state was calculated to be = 95.6% ± 0.39. There was no evidence for capacity-limited, or concentration-dependent binding in the clinically relevant range of plasma concentrations.

5.7 SAFETY EVALUATIONS

5.7.1 Laboratory Evaluations:

Standard laboratory evaluations were conducted at Screening, on Days 1, 7, 14, 21, 28 and 37 of the Treatment period, and at the time of Study Discharge. A treatment emergent abnormal value (TEAV) for a laboratory parameter was defined as a value that was below the lower limit of the normal range post-dose but was not below it prior to dose administration, or a value that was above the upper limit of the normal range post-dose, but was not above it prior to dose administration.

The number of TEAVs noted during the course of this study are listed by dose-group in TABLE 4. For reasons which are unclear, almost all study participants presented with minor changes in their sodium and chloride levels which were just outside the

normal range (1 or 2 g/dL). None of these changes were considered by the Investigator to be clinically significant. Investigators comments on these changes and on each additional TEAV is presented in DATA LISTING 9.

A summary of all the clinical laboratory values obtained for each subject during the study is presented in DATA LISTING 6.

5.7.2 Routine Physical Examinations:

General well being was assessed by a brief evaluation of the head, eyes, ears, nose, throat and other physical conditions of note upon admission to the study site for the Treatment period. No abnormal findings in general physical well being were noted for any subject.

A tabulation of Routine Physical Examination results is provided in DATA LISTING 4.

5.7.3 Electrocardiogram

All 16 subjects enrolled in the study had an ECG examination at the time of Screening and again at the time of their Final discharge. At both Screening and Final Discharge, several subjects were noted to have minor abnormalities such as left or right axial deviations or sinus bradycardia. All of these abnormalities were judged by the Investigator to be not clinically significant. No significant changes in ECG patterns were noted for any subject at the conclusion of the study. There were mild variations in heart rate between Screening and Final discharge for all of the subjects, but there was no discernible trend to these fluctuations and they were judged by the Investigator to be not clinically relevant. The electrocardiogram data is presented in DATA LISTING 5.

5.7.4 Vital Signs

Vital signs for all subjects (pulse, BP, respirations, temperature and weight), during the course of the treatment period are presented in DATA LISTING 11. The mean changes from baseline for radial pulse rate, systolic and diastolic blood pressure for each treatment group are presented in TABLE 3. No clinically significant changes in vital sign parameters were noted for any subject during any Treatment period.

5.7.5 Adverse Events

No serious or unexpected adverse events were reported during this study. All events reported were transient and mild to moderate in severity, and all were considered "not-serious" by the Investigator.

All reported adverse events are presented in DATA LISTING 13.

Those events which took place after dose administration were classified as Treatment Emergent Signs and Symptoms (TESS) and are summarized by dose-group and event in TABLE 5.

PHARMACOKINETICS

Table 11: Summary Table, Pharmacokinetics Parameters - Days 1, 7, 14, 21 and 28

		$AUC_{\tau,ss}$, ng·hr/ml					$AUC_{0-\infty}$, ng·hr/ml
Dose(mg)	Study Day	1	7	14	21	28	28
5	Mean	89.7	473.8	576.8	606.1	634.8	2889.3
	SD	23.5	45.4	70.5	68.7	92.2	751.6
10	Mean	89.2	421.1	1029.3	1139.6	1127.8	5051.9
	SD	12.4	59.0	152.3	213.0	195.9	1613.6
		Cl_{ss}, L/hr					
Dose(mg)	Study Day	1	7	14	21	28	
5	Mean				8.34	8.02	
	SD				0.90	1.12	
10	Mean				9.02	9.09	
	SD				1.57	1.47	
		λ_z, hr^{-1}	$t_{1/2}$, hr	V_z, L	Accumulation Ratio	Accumulation Predicted	
Dose(mg)	Study Day	28	28	28			
5	Mean	0.009708	72.71	827.95	7.18	4.89	
	SD	0.001415	10.56	59.22	2.37	0.63	
10	Mean	0.009620	73.48	950.79	6.16	4.94	
	SD	0.001366	11.82	133.76	1.06	0.71	
		$C_{max,ss}$, ng/ml					
Dose(mg)	Study Day	1	7	14	21	28	
5	Mean	7.4	24.8	30.1	31.0	34.1	
	SD	1.4	2.7	3.5	3.1	7.3	
10	Mean	6.8	21.9	56.8	60.6	60.5	
	SD	1.3	2.3	9.1	8.6	10.0	
		$T_{max,ss}$, hr					
Dose(mg)	Study Day	1	7	14	21	28	
5	Mean	5.0	3.6	3.4	3.6	3.0	
	SD	2.1	1.2	0.7	1.1	1.4	
10	Mean	4.9	3.7	3.5	3.1	3.9	
	SD	2.2	0.5	0.8	0.6	1.0	
		DF%					
Dose(mg)	Study Day	1	7	14	21	28	
5	Mean	95.3	44.0	41.0	39.9	47.4	
	SD	36.0	7.2	8.3	12.7	19.5	
10	Mean	86.6	45.1	52.3	46.4	46.8	
	SD	34.9	7.6	15.8	14.4	7.3	

PHARMACOKINETICS

Table 9. Pharmacokinetic Parameters for E2020 during Multiple-Dose Administration

Dose (mg)	Study Day	AUC (ng·h/mL) 1	7	14	21	28	DAY28 AUC	CL/F	$t_{1/2}$	C_{max}	t_{max}
5	Mean	89.7	473.8	576.8	606.1	634.8	2889.3	47.4	72.7	8.02	828.0
	SD	23.5	45.4	70.5	68.7	92.2	751.6	19.5	10.6	1.12	59.2
10	Mean	89.2	421.1	1029.3	1139.6	1127.8	5051.9	46.8	73.5	9.09	950.8
	SD	12.4	59.0	152.3	213.0	195.9	1613.6	7.3	11.8	1.47	133.8

Table 10. Accumulation of E2020: Predicted and Observed

Dose (mg)		Accumulation Ratio Predicted	Observed	Days to Steady-State
5	Mean	4.89	7.18	15.15
	SD	0.63	2.37	2.20
10	Mean	4.94	6.16	15.31
	SD	0.71	1.06	2.46

E2020 Pharmacodynamic Model Parameters for AChE Inhibition

			Model Predicted Values	
Study	Duration[a]	n[b]	E_{max}, % Inhibition	EC_{50}, ng/ml
E2020-A001-003	28 days	12	101.3	16.7
E2020-A001-011	28 days	16	109.8	18.2
E2020-A001-301	12 weeks	291	100.8	15.6
E2020-A001-302	24 weeks	291	98.4	13.4

[a]Maximum number consecutive doses
[b]Number of subjects contributing at one least point

Mean ± SD Observed Pharmacodynamic Parameters for E2020

		Observed Values		
Study	Dose	E_{min} %	E_{max} %	E_{ss} %
E2020-A001-003	5 mg	49.7 ± 7.7	64.2 ± 5.5	55.9 ± 5.5
	10 mg	67.2 ± 12.4	77.9 ± 8.1	72.3 ± 9.5
E2020-A001-011	5 mg	62.2 ± 5.8	71.8 ± 4.3	65.3 ± 5.2
	10 mg	74.7 ± 4.4	83.6 ± 1.9	77.8 ± 3.0

Table 4

Treatment Emergent Abnormal Laboratory Values

Number (%) of Subjects, by Treatment Group

Laboratory Test	E2020 (5 mg) (8)	E2020 (10 mg) (8)	Total (16)
Clinical Chemistry			
Albumin	1 (12.50%)	1 (12.50%)	2 (12.50%)
Alkaline Phosphatase	0 (0.00%)	1 (12.50%)	1 (6.25%)
ALT (SGPT)	2 (25.00%)	1 (12.50%)	3 (18.75%)
AST (SGOT)	1 (12.50%)	0 (0.00%)	1 (6.25%)
BUN	1 (12.50%)	0 (0.00%)	1 (6.25%)
Calcium	0 (0.00%)	0 (0.00%)	0 (0.00%)
Chloride	7 (87.50%)	8 (100.00%)	15 (93.75%)
Cholesterol	2 (25.00%)	1 (12.50%)	3 (18.75%)
Creatine Kinase (CK)	0 (0.00%)	1 (12.50%)	1 (6.25%)
CK-MB	0 (0.00%)	0 (0.00%)	0 (0.00%)
Creatinine	2 (25.00%)	1 (12.50%)	3 (18.75%)
Glucose	0 (0.00%)	2 (25.00%)	2 (12.50%)
Phosphorus	1 (12.50%)	0 (0.00%)	1 (6.25%)
LDH	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total Protein	1 (12.50%)	2 (25.00%)	3 (18.75%)
Total Bilirubin	0 (0.00%)	0 (0.00%)	0 (0.00%)
Sodium	8 (100.00%)	6 (75.00%)	14 (87.50%)
Potassium	2 (25.00%)	1 (12.50%)	3 (18.75%)
Hematology			
RBC	0 (0.00%)	0 (0.00%)	0 (0.00%)
Hemoglobin	0 (0.00%)	0 (0.00%)	0 (0.00%)
Hematocrit	1 (12.50%)	1 (12.50%)	2 (12.50%)
MCV	0 (0.00%)	1 (12.50%)	1 (6.25%)
MCH	0 (0.00%)	0 (0.00%)	0 (0.00%)
MCHC	3 (37.50%)	2 (25.00%)	5 (31.25%)
WBC	1 (12.50%)	0 (0.00%)	1 (6.25%)
Bands	0 (0.00%)	0 (0.00%)	0 (0.00%)
Neutrophils	1 (12.50%)	0 (0.00%)	1 (6.25%)
Lymphocytes	1 (12.50%)	0 (0.00%)	1 (6.25%)
Monocytes	1 (12.50%)	0 (0.00%)	1 (6.25%)
Eosinophils	0 (0.00%)	0 (0.00%)	0 (0.00%)
Basophils	0 (0.00%)	1 (12.50%)	1 (6.25%)
not used	0 (0.00%)	0 (0.00%)	0 (0.00%)
Platelet Count	1 (12.50%)	1 (12.50%)	2 (12.50%)
RBC Morphology	0 (0.00%)	0 (0.00%)	0 (0.00%)
WBC Morphology	0 (0.00%)	0 (0.00%)	0 (0.00%)

Treatment Emergent Abnormal Value: Value at discharge is outside normal range and different than Screening.
Source Data: Data Listing 6

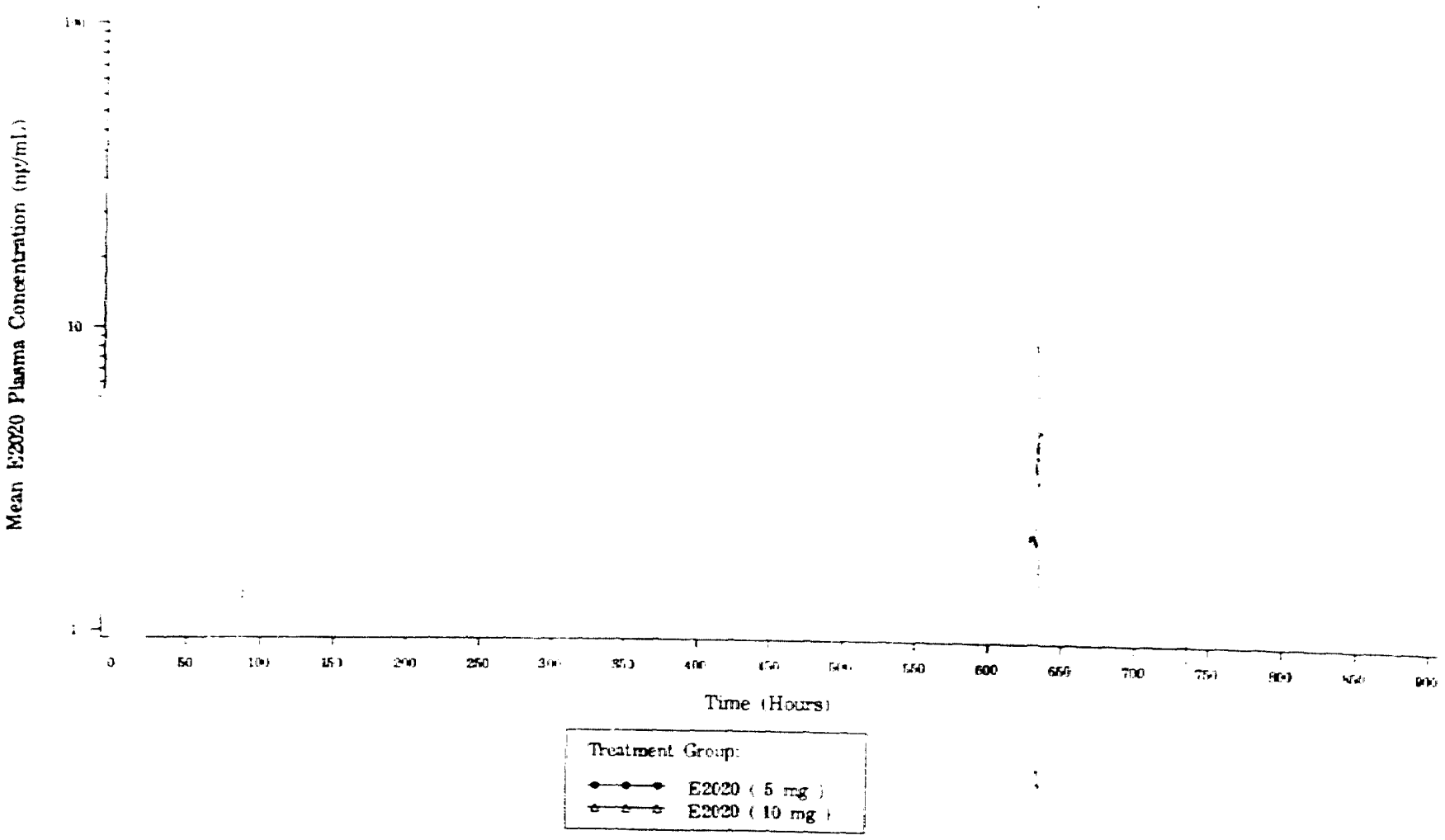
Mean (SD) E2020 Pharmacokinetic Parameters in the Morning Versus the Evening (Day 21)

	n	AUC_{0-24}, ng·hr/mL	C_{max}, ng/mL	t_{max}, hr	$t_{1/2}$, hr	CL/F, L/hr/Kg	V/F, L/Kg
E2020-A001-002 (Morning)	6	546.5 (52.6	3 (4.2	3.2	(7.3, 9.9)	0.125 (0.016	12.3
E2020-A001-011 (Evening)	7	606.1 (68.7	3 (3.1	3	72.7 (10.6)[a]	0.115 (0.02	11.8

[a]Day 28 parameter.

Figure II
E2020 – A001 – 011

E2020 Plasma Concentration (ng/mL)



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100

PHARMACOKINETICS

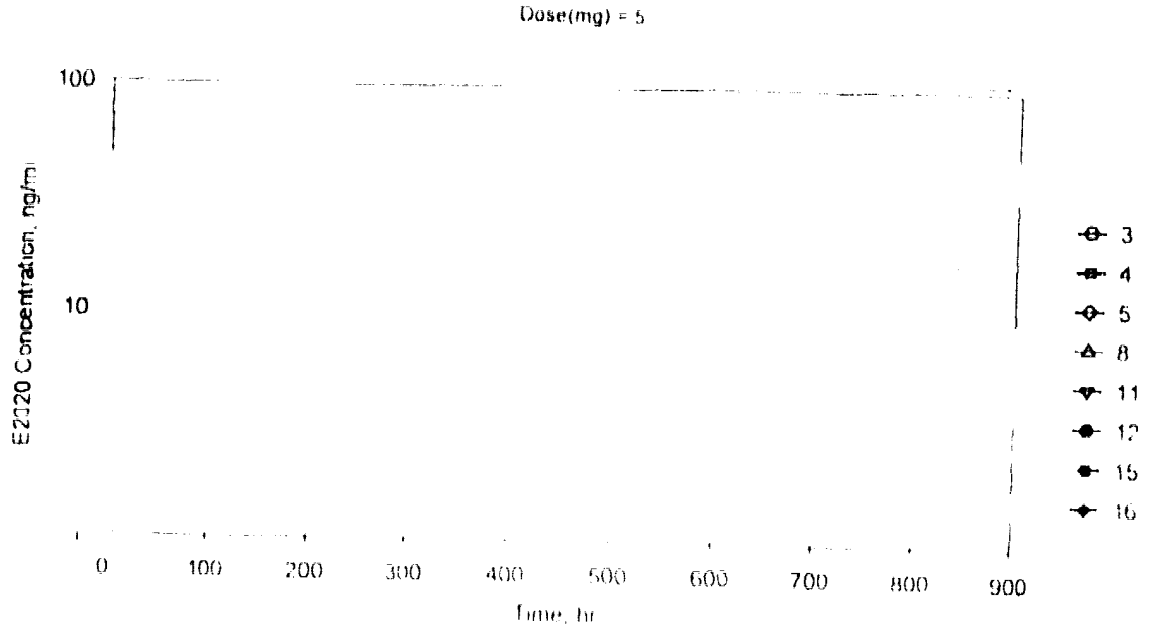


Figure III - E2020 Plasma Concentrations Versus Time - All Subjects - 5mg Dose.

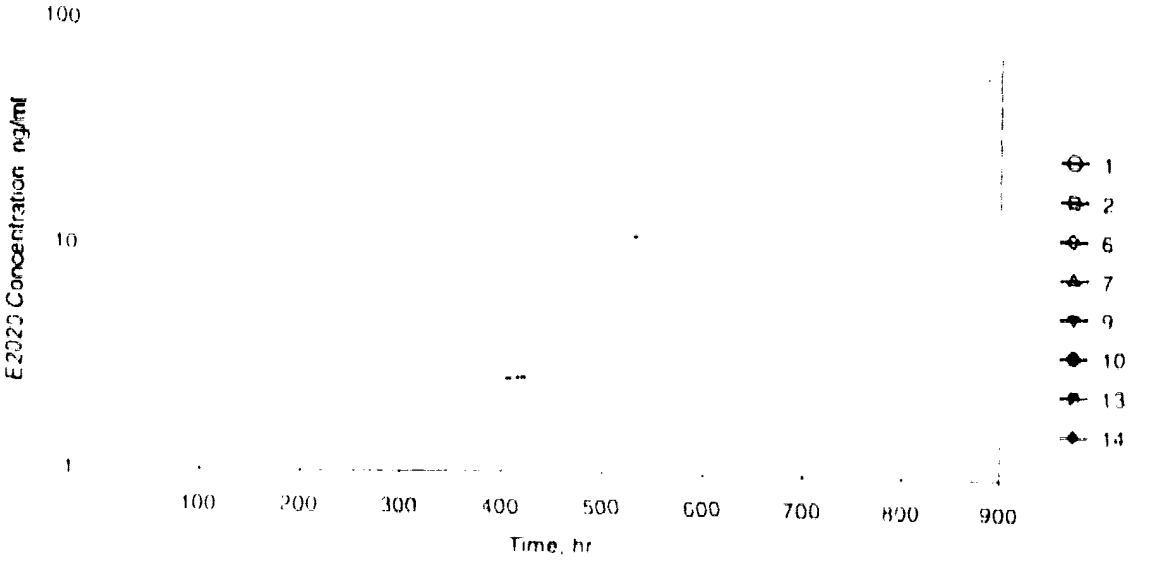


Figure IV - E2020 Plasma Concentration versus Time - All Subjects - 10mg Dose.

PHARMACODYNAMICS

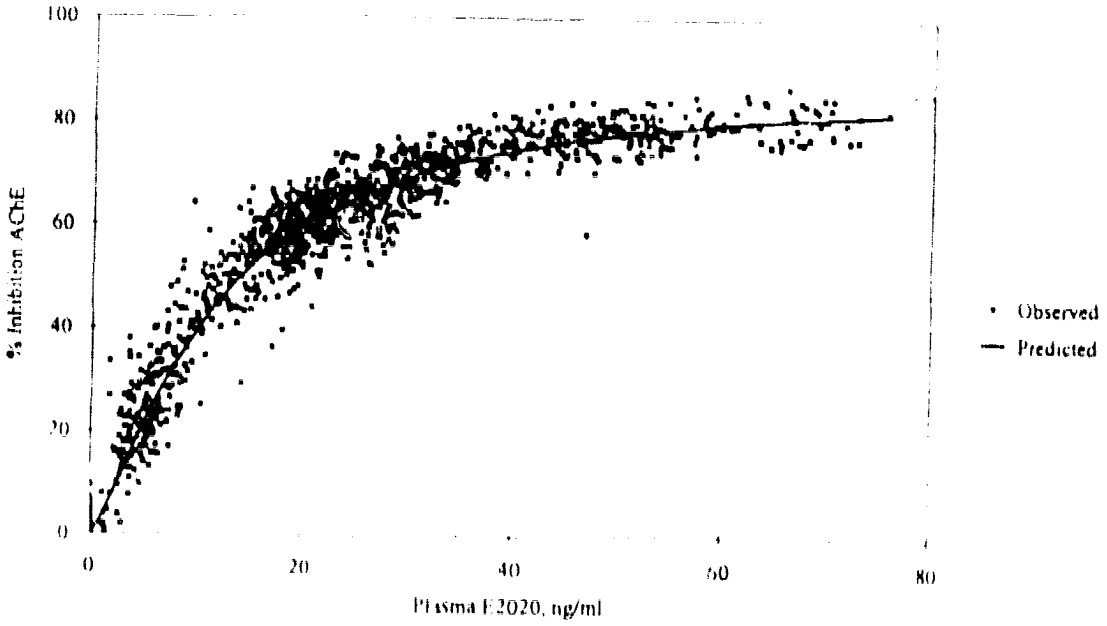
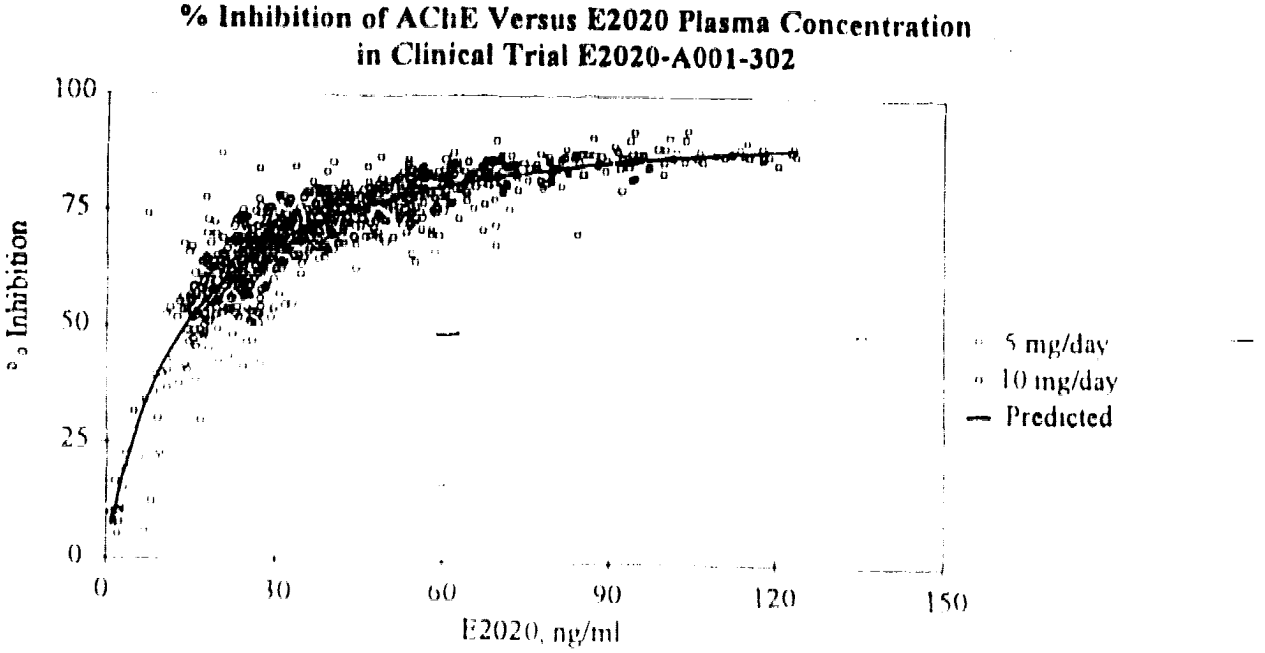


Figure VII - Percent inhibition of red blood cell acetylcholinesterase (AChE) versus plasma E2020 concetration. All points for which reported values of both measures were present were included in the analysis. The solid line was generated by fitting a sigmoidal E_{max} model to the pooled data.



PHARMACOKINETICS

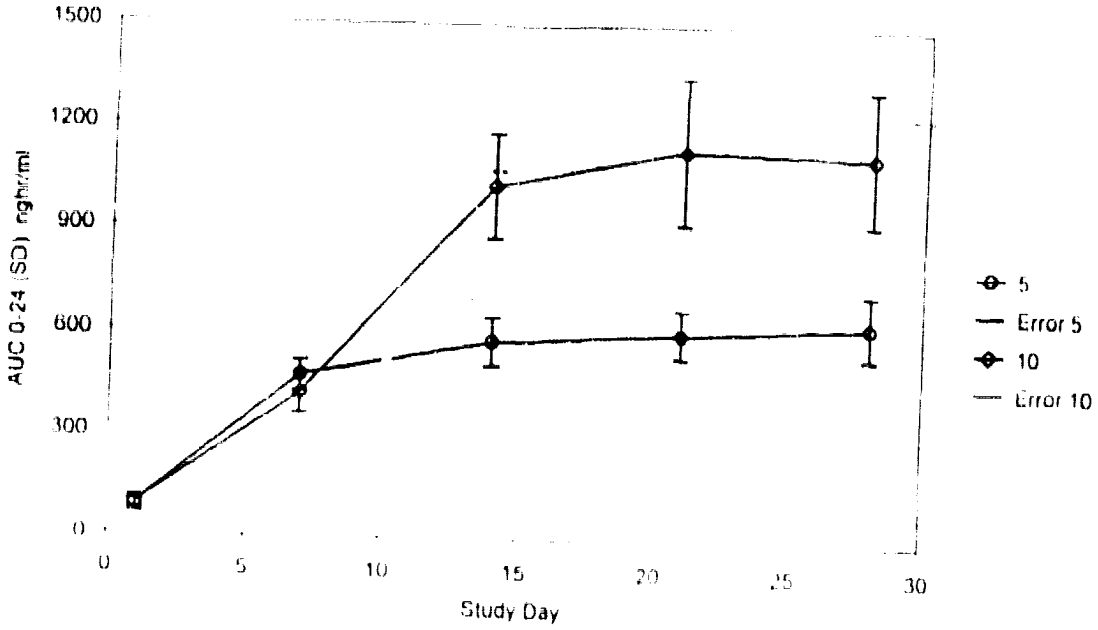
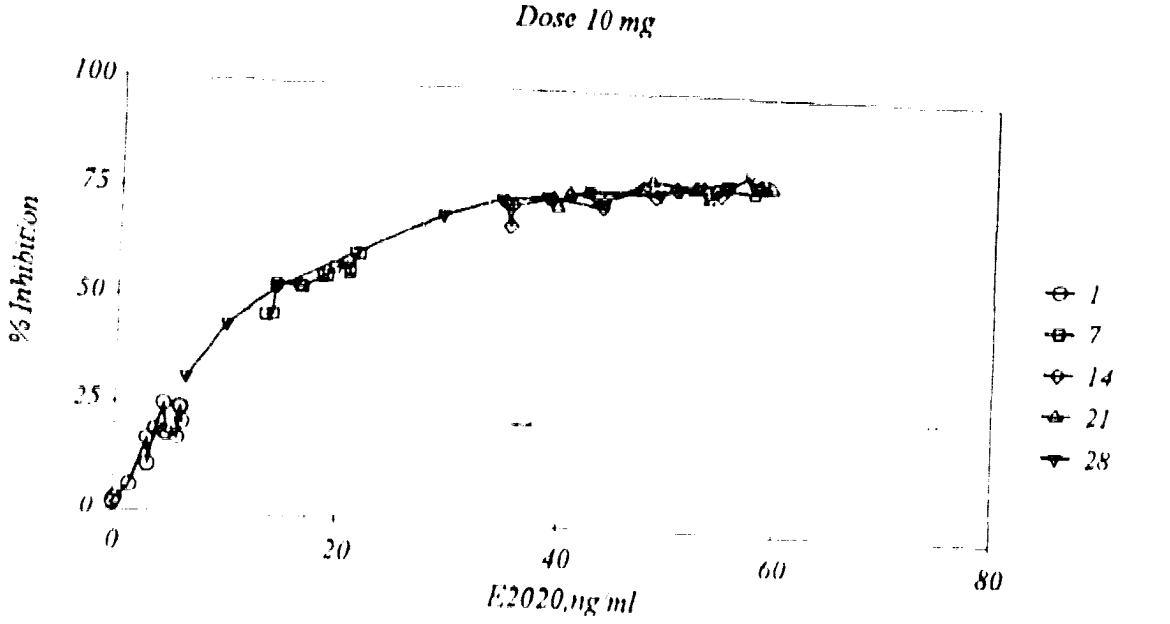


Figure V - $AUC_{(0-24)}$ versus Study Day (AUC ± SD) for the 24 hour evaluation period on Days 1, 7, 14, 21, and 28.



PHARMACODYNAMICS

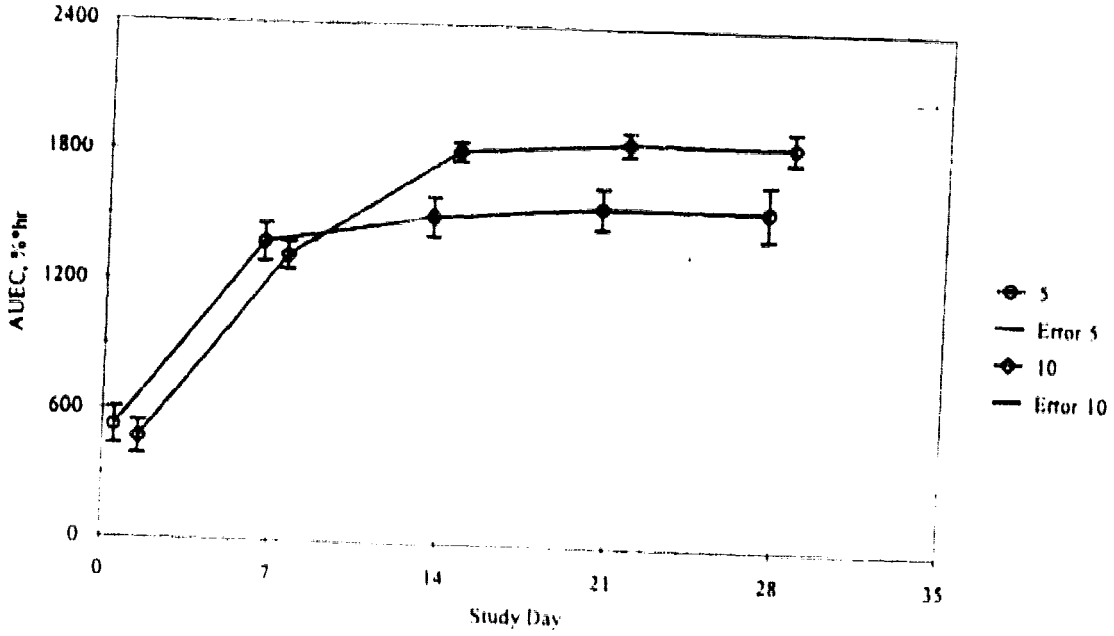


Figure VIII - Average Area under the Effect curve (AUEC) on Days 1, 7, 14, 21, and 28.

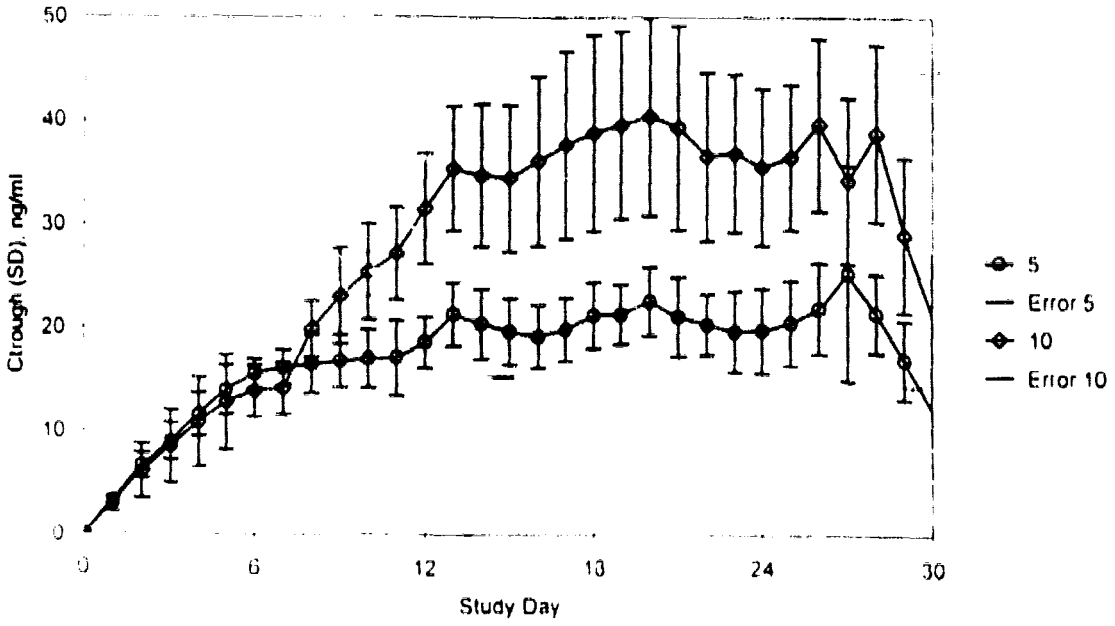


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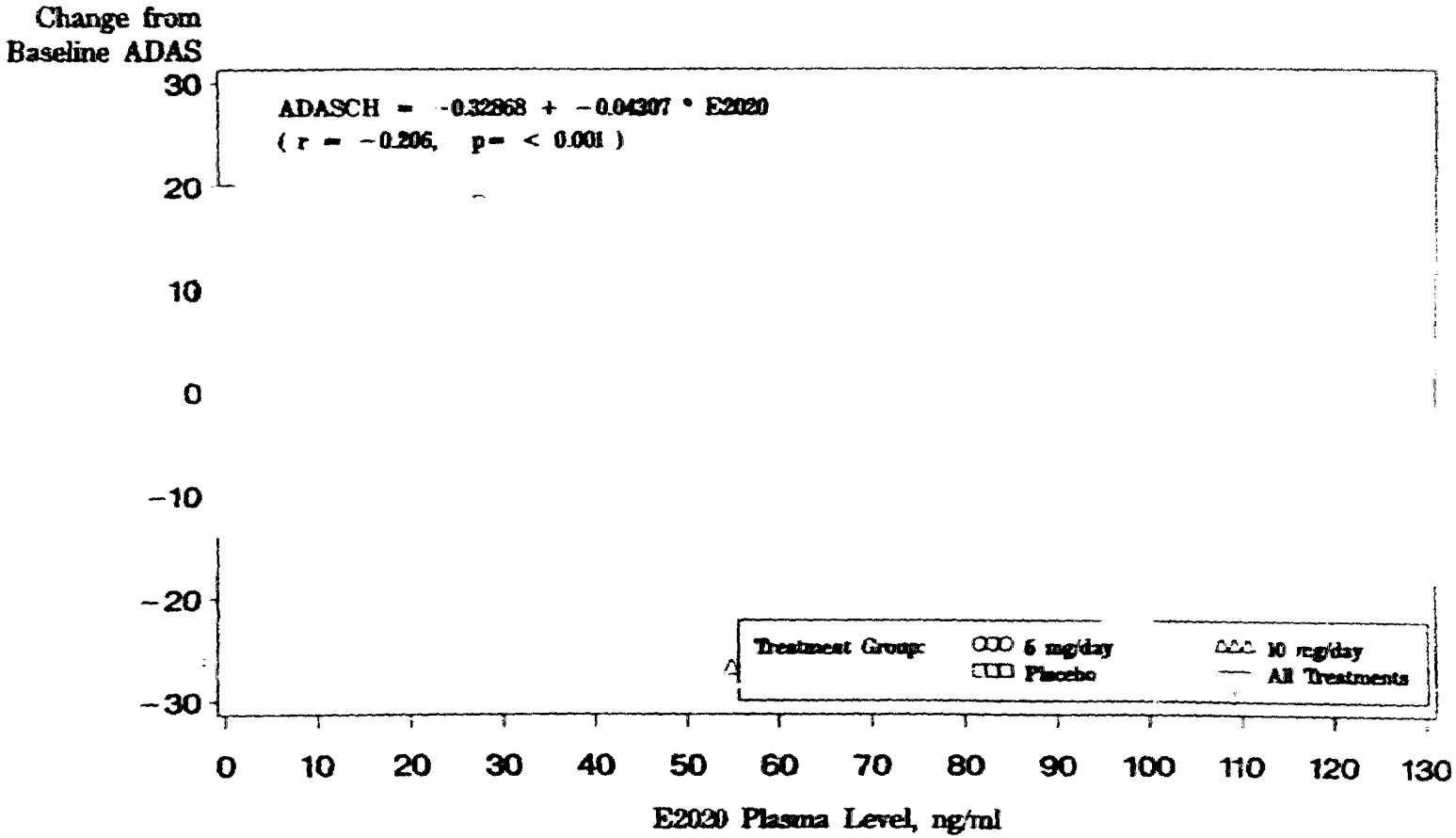
Figure VI - Average trough concentrations of E2020 on Days 1 through 29.

66

Figure 20.1
Change from Baseline ADAS vs. E2020 Plasma Level
Endpoint



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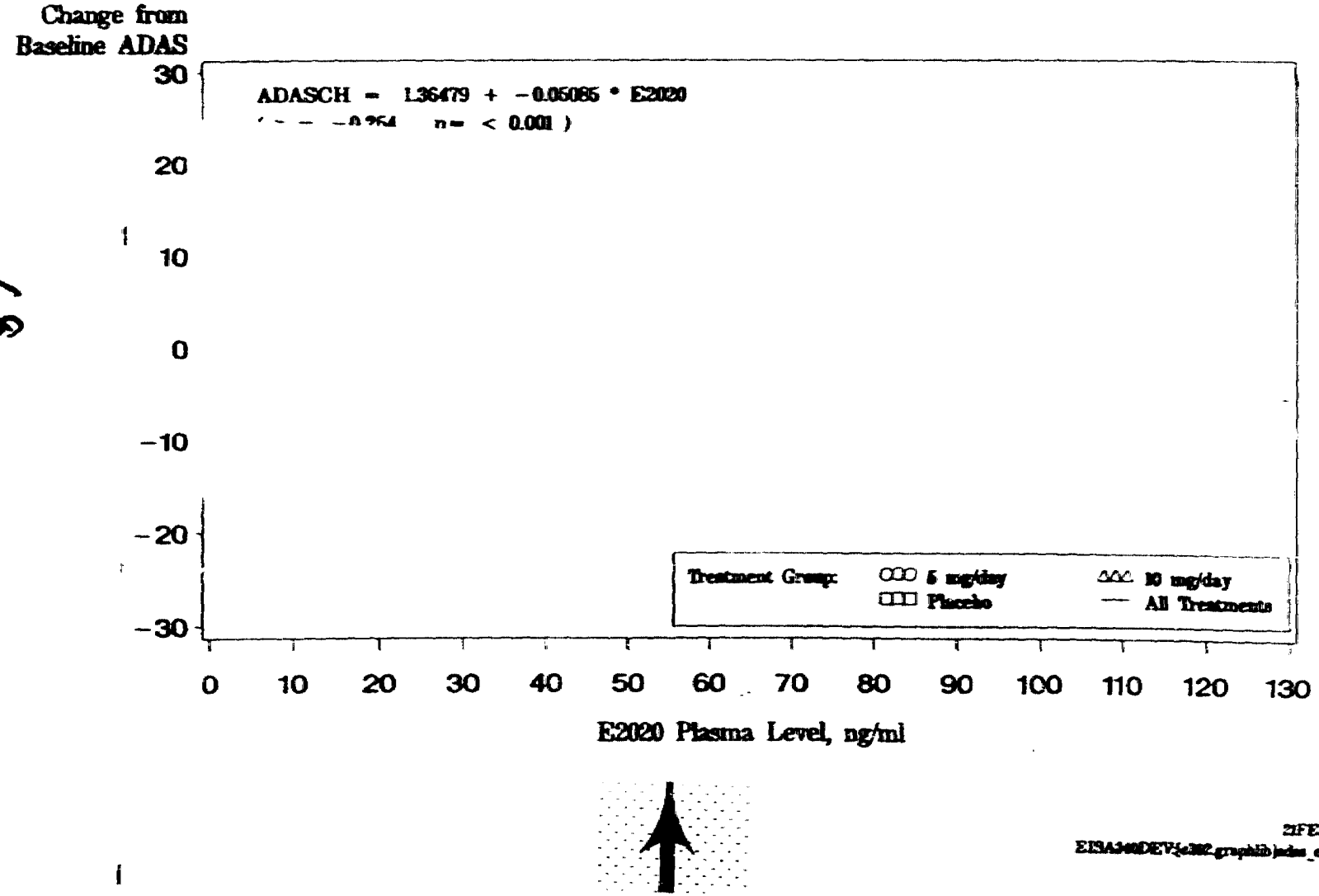
EISAI AMERICA, INC.
STUDY E2020-A001-302

Figure 20.2
Change from Baseline ADAS vs. E2020 Plasma Level
Endpoint



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METABOLISM - IN VIVO

CLINICAL STUDY SYNOPSIS

Title: A Single-Dose Study of the Metabolism and Elimination of ^{14}C-E2020, An Acetylcholinesterase Inhibitor, in Healthy Volunteers. Study #: E2020-A001-004

Investigator:

Objective: The objective of this study was to investigate the metabolism and elimination of E2020 in humans, following the administration of a single 5-mg (liquid) oral dose containing a mixture of unlabeled and ^{14}C-labeled E2020.

Study Design: This was an open-label, non-randomized, single-dose study which was conducted at a single investigational site.

Study Population: A total of 8 healthy, male volunteers were enrolled into this study and all 8 volunteers completed the study without incident. All participants were Caucasion and ranged in age from 19 to 43 years (mean ± SE: 27.0 ± 3.2 years) and in weight from 73.5 to 85.0 kg (mean ± SE: 78.6 ± 1.4 kg).

Drug Administration: The subjects in this study received a single, 5-mg oral dose (in a 20-ml aqueous solution) containing 4.62 mg of E2020 and 0.38 mg (50 µCi) of ^{14}C-labeled E2020.

Methods *(Clinical)*: Volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to the start of the study. Subjects were admitted to the study site on the evening of day 0, at least 12 hours prior to drug administration. Upon admission, a routine physical examination was conducted. Urine specimens for drug use/abuse screening were collected and baseline urine and fecal analytical samples were also collected. A light snack was served 10 hours prior to drug administration, after which an absolute fast, from both food and fluids (except water), was maintained. The following morning, prior to drug administration, clinical laboratory and analytical samples were collected. Subjects then received a single 5 mg (liquid) dose of E2020 (containing a mixture of unlabeled and ^{14}C-labeled E2020), followed by 250 ml of tap water. Following drug administration, blood, urine and fecal samples for analytical determinations were collected at specified times and intervals for 10 days. Vital signs were monitored at 8-hour intervals and monitoring for adverse experiences was conducted continuously. Subjects were discharged from the investigational site on the morning of day 11, following a 240-hour blood sample and a final discharge evaluation. Total in-house time was approximately 256 hours.

Methods *(Analytical)*: Characterization of ^{14}C-E2020 metabolism and elimination was done by analyzing blood, urine and fecal samples collected over the 10-day period following drug administration. Each collected sample was assayed for total radioactivity, and aliquots from specified timepoints and/or pooled samples were assayed for the presence of E2020 metabolites. In addition, aliquots from each blood sample were also assayed for total E2020 concentration by high-performance liquid chromatography (HPLC).

Pharmacokinetic (PK) parameters were calculated for plasma E2020 concentrations and for total plasma radioactivity (^{14}C-E2020) using standard techniques. The following parameters were calculated: Cmax, tmax, $AUC_{(0-144)}$, $AUC_{(0-\infty)}$, elimination rate constant (λ_z), Clearance (Cl/F), Volume of distribution ($V\lambda_z$ /F) and $t_{1/2}$.

Results:

Absorption, Distribution and Metabolism: Plasma radioactivity (expressed as "ng E2020 equivalents/ml"), maximized in the 6 to 8 hour time interval at 10.09 ng equiv./ml. E2020, which was the predominant compound during all plasma time intervals, also maximized in concentration during this time (4.07 ng/ml). In general, E2020 accounted for about 25% of the recovered radioactivity in each time interval, with the exception of the Cmax interval, where it accounted for about 40% of the total radioactive residue.

In general, the recovered residue in plasma showed higher levels of the hydroxylated metabolites M1 and M2 than their glucuronide (or sulfate) conjugates M11 and M12 respectively. M6 was a significant metabolite in the first four-hour sample pool but steadily decreased during the following intervals. In contrast, the hydrolysis product M4, was never present in significant concentrations for any time interval in plasma (≤ 0.1 ng equiv./ml).

Elimination: Recovery of radioactivity in subject samples averaged 72% of the administered dose. Recovery in urine (57%) was significantly greater than recovery in feces (15%).

In urine, the parent compound E2020 accounted for 17% of the recovered dose on average. The major metabolite was the hydrolysis product, M4 (6%), followed by the glucuronidated conjugates M11 and M12 (5% and 2% of the dose, respectively). The N-oxidation product M6 accounted for 2% of the dose. The hydroxylation metabolites M1 and M2 averaged less than 2%.

In feces, approximately 5% of the recovered dose was unextractable (3% remained as post-extraction solid, 2% was highly polar and remained in the aqueous phase). E2020 accounted for 1% of the dose. Of the extractable metabolites, the hydroxylation metabolites M1 and M2 were the most abundant (0.7% and 0.6% of the dose, respectively). Glucuronidated metabolites M11 and M12 accounted for less than 0.2%. M4 and M6 accounted for 0.5% and 0.2% of the dose, respectively.

A schematic representation of the structures of the E2020 metabolites as well as the proposed metabolic pathway for E2020 is shown in the Conclusion section of this Synopsis and in Section 5.4 of the Results.

Pharmacokinetics: Several of the pharmacokinetic parameters calculated for this study are summarized in the table below.

As shown in the table, the t_{max} values for E2020 and for ^{14}C-E2020 (total radioactivity) were similar. The C_{max} values, however, were markedly greater for ^{14}C-E2020 than for E2020, demonstrating the presence of E2020 metabolites. In a similar fashion, both AUC values were also markedly greater for ^{14}C-E2020 than for E2020. The ratio of E2020, $AUC_{(0-144)}$ / Total ^{14}C-E2020, $AUC_{(0-144)}$, was 53.7%.

Comparison of Pharmacokinetic Parameters (Mean ± SE)

	t_{max} (hrs)	C_{max} (ng/ml)	$AUC_{(0-144)}$ (ng*hr/ml)	$AUC_{(0-\infty)}$ (ng*hr/ml)	$t_{1/2}$ (hrs)
E2020	4.4 (0.5)	7.2 (0.6)	355.0 (20.7)	507.4 (31.8)	78.4 (5.5)
^{14}C-E2020	4.6 (0.6)	11.4 (0.8)	663.5 (23.0)	897.3 (36.1)	70.6 (3.6)

Safety: No serious or unexpected adverse experiences occurred during the course of this study. Treatment Emergent Signs and Symptoms (TESS) were transient and all were mild or moderate in severity.

No clinically significant, treatment emergent abnormal laboratory values (TEAV's) were observed during the course of this study.

Conclusions:

The predominant route of elimination for E2020 and it's metabolites appears to be renal, with 79% of the recovered dose being found in the urine. The remaining 21% of the recovered dose was found in the feces.

The parent compound E2020 is the predominant elimination product in urine and feces. The major metabolites of E2020 which are eliminated include M1 and M2 (via O-dealkylation and hydroxylation), M11 and M12 (via glucuronidation of M1 and M2, respectively), M4 (via hydrolysis) and M6 (via N-oxidation). *(continued)*

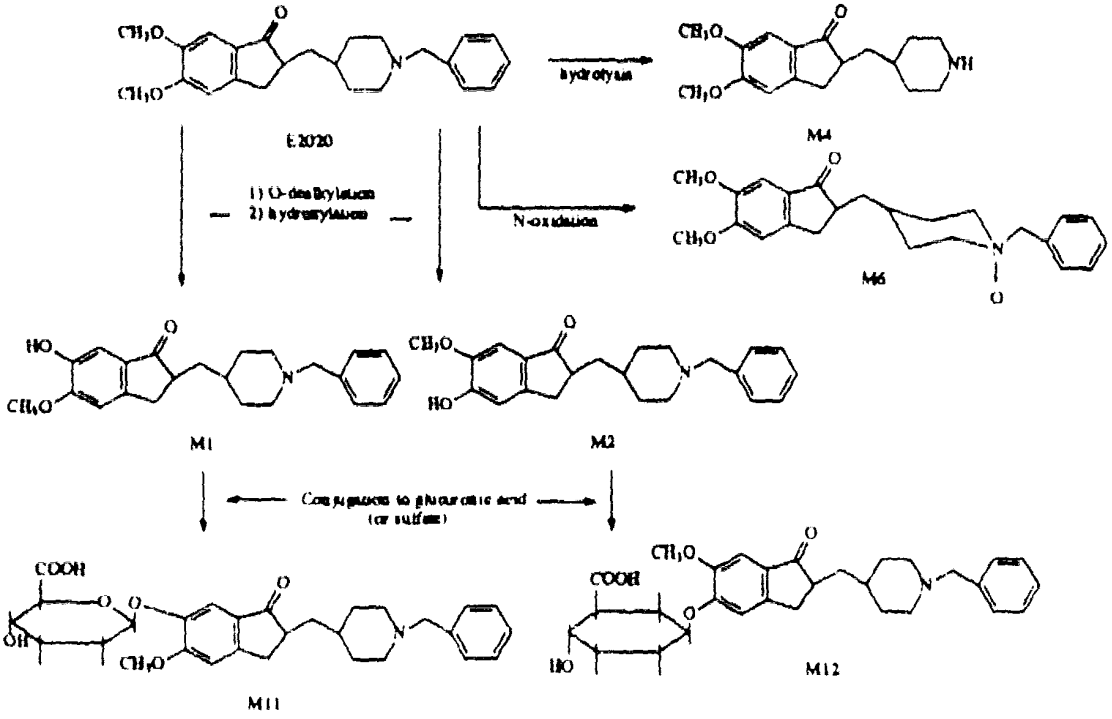
The proposed metabolic pathways for E2020 based upon the results of this study are as follows:

1) O-dealkylation followed by hydroxylation to metabolites M1 and M2, with partial subsequent glucuronidation to metabolites M11 and M12 respectively.

2) Hydrolysis to metabolite M4.

3) N-oxidation to metabolite M6.

The Proposed Metabolic Pathway for E2020:



5.4.4 Summary - E2020 Metabolite Data

A summary of the average totals (average of 8 subjects over all time intervals), for E2020 and each metabolite is shown below in the table below. Summary values are presented in decreasing order (from greatest amount recovered to least) for each matrix. Plasma values represent the average total amount recovered *cumulatively* from all five time intervals. As noted in Section 4.3.2 of this report, original dpm values were converted to "ng E2020 equivalents/ml" and are presented as such. Values for urine and feces represent the average total amount of E2020 and each metabolite recovered over all time periods assayed. Values are presented as "% of Total ^{14}C-E2020 Dose Recovered" for these matrices.

Table II : Summary of E2020 Metabolite Data - The numbers shown represent cumulative totals from the 10-day collection period.

Plasma (ng E2020 equivalents/ml)		Urine (% of Recovered Dose)		Feces (% of Recovered Dose)	
E2020	10.0	E2020	16.9%	E2020	1.1%
M1	3.5	M4	6.1%	M1	.66%
M6	2.8	M11	4.9%	M2	.59%
M2	2.3	M12	2.1%	M4	.46%
M12	1.0	M6	1.9%	M6	.24%
M11	.66	M1	1.6%	M11	.14%
M4	.07	M2	1.1%	M12	.08%
% Aqueous	4.1	% Aqueous	13.9%	% Aqueous	1.4%
TLC Origin	0.0	TLC Origin	0.0%	TLC Origin	1.6%

5.5 PHARMACOKINETICS

Pharmacokinetic parameters were calculated for both total plasma E2020 (as determined by , and for ^{14}C-E2020 (as calculated by total radioactivity). The following parameters were calculated: Cmax, tmax, $AUC_{(0-144)}$, $AUC_{(0-\infty)}$, terminal disposition constant (λ_z), clearance (Cl/F), volume of distribution ($V_{\lambda z}$/F) and $t_{1/2}$. In addition, the ratio of E2020, AUC_{0-144} / Total ^{14}C-E2020, AUC_{0-144}, was also calculated.

Mean plasma E2020 concentrations (as determined by are summarized by timepoint in TABLE 7. E2020 plasma concentration data for each subject is presented in DATA LISTING 18 and mean ^{14}C-E2020 concentrations, expressed as "ng E2020

Excretion of E2020 and Metabolites Expressed as % of Total Dose

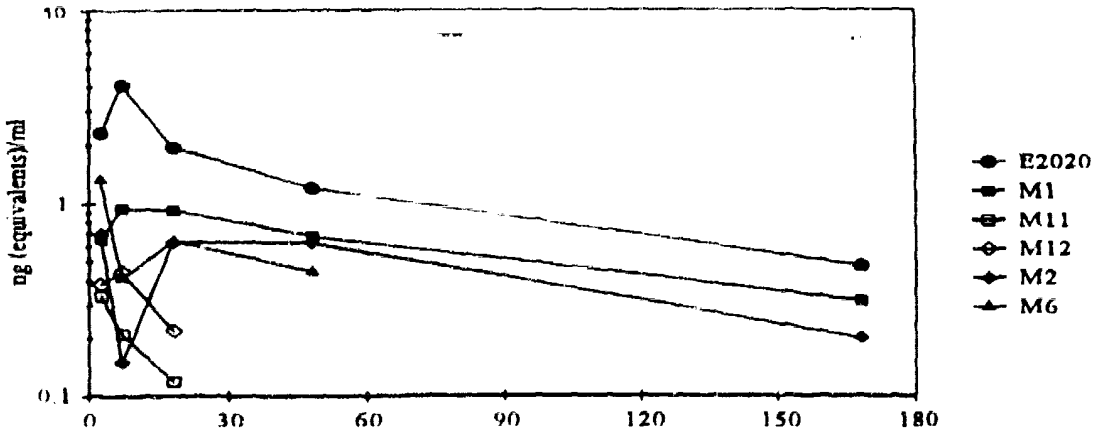
		Putative Isozyme	Protocol Number A001-004[a]	J081-001	J081-003	J081-004
	Urinary Excretion					
E2020			16.9 ± 7.0	10.6 ± 3.5	11.77 ± 4.96	10.69 ± 10.84
M1	6-O-desmethyl-E2020	CYP2D6	1.5 ± 0.6	0.03 ± 0.04	nd	0.01 ± 0.03
M11	M1 - Glucuronide		4.9 ± 2.5	9.7 ± 6.1	6.64 ± 2.21	3.74 ± 1.77
M2	5-O-desmethyl-E2020	CYP2D6	1.1 ± 0.6	0.01 ± 0.01	0.09 ± 0.23	0.01 ± 0.02
M12	M2 - Glucuronide		2.1 ± 0.8	3.06 ± 2.26	1.83 ± 0.58	1.72 ± 1.79
M4	N-debenzyl-E2020	CYP3A4	6.1 ± 1.8	12.5 ± 2.7	13.67 ± 3.66	6.76 ± 6.02
M6	E2020-cis-N-oxide	CYP3A4	1.9 ± 0.8	0.27 ± 0.19	nd	nd
Total Radioactivity Excreted in Urine			57.0 ± 3.5	nd	nd	nd
Total Radioactivity Excreted in Feces			14.5 ± 5.8	nd	nd	nd
Mass Balance			71.5	nd	nd	nd

[a]Determined from ^{14}C-labeled E2020

Mean ± SD Pharmacokinetic Parameters Specific for E2020 Plasma Concentrations and Total Plasma ^{14}C-Radioactivity

	$AUC_{0-\infty}$, ng·hr/mL	C_{max}, ng/mL
Specific E2020	507.4 ± 89.9	7.2 ± 1.7
Total ^{14}C-Radioactivity (expressed as E2020 ng equivalents)	897.3 ± 102.1	11.4 ± 2.3

Disposition Profiles of E2020 Metabolites in Plasma Following Oral Administration of 5 mg ^{14}C-E2020



Appendix VI
Pharmacokinetic Parameters by Subject

	SUBJECT NUMBER	WEIGHT (KG)	Cmax (ng/mL)	tmax (hrs.)	AUC(0-144) (ng hr/mL)	AUC(0-inf) (ng hr/mL)	CL/F (L/hr/kg)	HALF LIFE (hrs.)	TERMINAL DISPOSITION CONSTANT(K) (Hour^-1)	V/F (L/kg)	Ratio (%)
14C-E2020 5mg	001										
	002										
	003										
	004										
	005										
	006										
	007										
	008										
E2020 5mg	001										
	002										
	003										
	004										
	005										
	006										
	007										
	008										

Ratio = 100 x Plasma E2020 AUC(0-144) / Total Radioactive E2020 AUC(0-144).

TABLE XXI. PLASMA: OBSERVED METABOLITES QUANTIFIED BY TIME INTERVAL AS THE AVERAGE CONCENTRATION (ng E2020 Equivalents/mL) OVER 8 SUBJECTS

COMPOUND MONITORED	Approx. Rf	PLASMA Average* of 8 subjects, (ng E2020 equiv./mL +/- Standard Deviation)									
		1,2,3,4 hrs		6,8 hrs		12,18,24 hrs		36,48,72 hrs		96-240 hrs	
		AVG. ng/mL	STDS	AVG. ng/mL	STDS	AVG. ng/mL	STDS	AVG. ng/mL	STDS	AVG. ng/mL	STDS
	TOTAL:	8.17	0.40	10.09	0.56	7.84	0.55	5.09	0.30	2.01	0.11
E2020	(Rf 89)	2.31	0.38	4.07	0.39	1.95	0.48	1.19	0.39	0.47	0.21
M1	(Rf 81)	0.65	0.34	0.94	0.49	0.93	0.35	0.67	0.23	0.31	0.14
M11 (as M1 post-hyd)[1]	(Rf 81)	0.33	0.51	0.21	0.44	0.12	0.34	BDL	BDL	BDL	BDL
M2	(Rf 75)	0.69	0.16	0.15	0.27	0.63	0.11	0.62	0.02	0.20	0.08
M12 (as M2 post-hyd)	(Rf 75)	0.38	0.21	0.44	0.42	0.22	0.38	BDL	BDL	BDL	BDL
M6	(Rf 72)	1.33	0.12	0.41	0.39	0.64	0.20	0.44	0.27	BDL	BDL
M4	(Rf 20)	0.07	0.05	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
UNK 1	(Rf 62)	BDL	BDL	0.06	0.17	0.15	0.43	BDL	BDL	BDL	BDL
UNK 2	(Rf 53)	0.34	0.24	0.60	0.37	0.16	0.10	BDL	BDL	BDL	BDL
UNK 3	(Rf 36)	0.20	0.12	0.26	0.16	BDL	BDL	BDL	BDL	BDL	BDL
UNK 4	(Rf 25)	BDL	BDL	BDL	BDL	0.86	0.53	0.85	0.52	0.45	0.28
UNK 5	(Rf 10)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
TLC Origin	(Rf 0)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
% Aqueous:		0.89	0.09	1.29	0.17	0.57	0.13	0.89	0.59	0.47	0.25
Mass Balance:		**7.23**	**0.61**	**8.51**	**0.63**	**6.24**	**0.37**	**4.67**	**0.52**	**2.02**	**0.18**

* Weighted average (6*(Pooled Subjects)+Subject 2 + Subject 4)/8 subjects total
ND = not detected. <5% total radioactive residue in chromatographed sample.
BDL = Below Detection Limit

TABLE III. URINE: OBSERVED METABOLITES QUANTIFIED BY TIME INTERVAL AS THE AVERAGE % DOSE OF 8 SUBJECTS

COMPOUND MONITORED	Approx. Rf	URINE, Average of 8 Subjects (% Dose +/- Standard Deviation)										
		0-8 hrs		8-24 hrs		24-48 hrs		48-96 hrs		96-240 hrs		
		% Dose	+/- SD	% Dose	+/- SD	% Dose	+/- SD	% Dose	+/- SD	% Dose	+/- SD	TOTAL % Dose
	TOTAL:	5.6%	1.4%	10.0%	0.8%	11.2%	1.3%	13.4%	1.6%	16.9%	3.2%	57.0%
E2020	(Rf 89)	1.7%	1.0%	2.9%	1.4%	3.7%	1.8%	4.1%	1.8%	4.5%	2.1%	16.9%
M1	(Rf 81)	0.1%	0.1%	0.2%	0.2%	0.1%	0.2%	0.4%	0.2%	0.7%	0.4%	1.5%
M1 (as M1 post-hyd)	(Rf 81)	0.6%	0.4%	1.0%	0.6%	1.0%	0.5%	1.0%	0.4%	1.3%	0.6%	4.9%
M2	(Rf 75)	BDL	0.1%	0.1%	0.1%	0.1%	0.2%	0.3%	0.1%	0.5%	0.3%	1.1%
M12 (as M2 post-hyd)	(Rf 75)	0.2%	0.2%	0.3%	0.3%	0.4%	0.3%	0.5%	0.2%	0.7%	0.2%	2.1%
M4	(Rf 20)	0.6%	0.3%	1.2%	0.4%	1.4%	0.5%	1.2%	0.5%	1.6%	0.5%	6.1%
M6	(Rf 72)	0.1%	0.1%	0.1%	0.1%	0.2%	0.3%	0.6%	0.3%	0.9%	0.4%	1.9%
UNK 1	(Rf 62)	0.1%	0.1%	0.2%	0.2%	0.1%	0.2%	BDL	0.1%	0.3%	0.2%	0.6%
UNK 2	(Rf 53)	0.1%	0.1%	0.5%	0.2%	0.4%	0.2%	0.1%	0.1%	0.4%	0.4%	1.5%
UNK 3	(Rf 36)	0.2%	0.2%	0.3%	0.4%	0.2%	0.3%	0.4%	0.2%	0.5%	0.2%	1.5%
UNK 4	(Rf 25)	0.1%	0.1%	BDL	0.1%	BDL	0.1%	0.1%	0.2%	0.1%	0.1%	0.2%
UNK 5	(Rf 10)	BDL	BDL	BDL	BDL	BDL	BDL	0.1%	0.1%	0.1%	0.1%	0.3%
UNK 6	(RF 87)	BDL	BDL	0.1%	0.1%	0.3%	0.3%	0.1%	0.3%	BDL	BDL	0.5%
TLC Origin	(Rf 0)	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
% Aqueous:		1.2%	0.4%	2.3%	0.6%	2.4%	0.6%	3.6%	0.5%	4.3%	1.1%	13.9%
Mass Balance:		5.1%	1.2%	9.4%	1.2%	10.6%	1.2%	12.6%	1.9%	15.8%	3.4%	53.4%

BDL = Below Detection Limit.

TABLE II. URINE: OBSERVED METABOLITES QUANTIFIED BY SUBJECT AS % DOSE OVER TEN DAYS AFTER DOSE ADMINISTRATION

COMPOUND MONITORED	Approx. Rf	URINE (% Dose by Subject) 1	2	3	4	5	6	7	8	Average	+/- SD
	TOTAL:									57.0%	3.5%
E2020	(Rf 89)									16.9%	7.0%
M1	(Rf 81)									1.5%	0.6%
M11 (as M post-hyd)	(Rf 81)									4.9%	2.5%
M2	(Rf 75)									1.1%	0.6%
M12 (as M2 post-hyd)	(Rf 75)									2.1%	0.8%
M4	(Rf 20)									6.1%	1.8%
M6	(Rf 72)									1.9%	0.8%
UNK 1	(Rf 62)									0.6%	0.3%
UNK 2	(Rf 53)									1.5%	0.6%
UNK 3	(Rf 36)									1.5%	1.1%
UNK 4	(Rf 25)									0.2%	0.2%
UNK 5	(Rf 10)									0.3%	0.2%
UNK 6	(RF 87)									0.5%	0.6%
TLC Origin	(Rf 0)									BDL	BDL
% Aqueous:										13.9%	1.1%
Mass Balance:										**53.4%**	3.9%

BDL = Below Detection Limit.

TABLE XII. FECES: OBSERVED METABOLITES QUANTIFIED BY TIME INTERVAL AS THE AVERAGE % DOSE OF 8 SUBJECTS

COMPOUND MONITORED	Approx. Rf	FECES, Average of 8 Subjects (% Dose +/- SD)					
		Day 2,3*		Day 5,6		Day 9,10	
		Avg % Dose	+/- SD	Avg % Dose	+/- SD	Avg % Dose	+/- SD
	TOTAL:	4.81%	2.34%	4.36%	2.71%	2.44%	1.25%
E2020	(Rf 89)	0.75%	0.54%	0.25%	0.15%	0.06%	0.04%
M1	(Rf 81)	0.39%	0.23%	0.23%	0.14%	0.04%	0.06%
M11 (as M1 post-hyd)	(Rf 81)	0.10%	0.09%	0.03%	0.04%	0.01%	0.02%
M2	(Rf 75)	0.30%	0.25%	0.23%	0.25%	0.06%	0.09%
M12 (as M2 post-hyd)	(Rf 75)	0.01%	0.02%	0.04%	0.04%	0.03%	0.05%
M4	(Rf 20)	0.24%	0.12%	0.15%	0.09%	0.07%	0.06%
M16	(Rf 72)	0.13%	0.10%	0.09%	0.14%	0.02%	0.04%
UNK 1	(Rf 62)	BDL	0.01%	BDL	BDL	BDL	BDL
UNK 2	(Rf 53)	0.01%	0.02%	0.01%	0.02%	BDL	BDL
UNK 3	(Rf 36)	BDL	0.01%	BDL	BDL	0.03%	0.04%
UNK 4	(Rf 25)	0.04%	0.12%	BDL	BDL	BDL	BDL
UNK 5	(Rf 10)	0.04%	0.05%	0.02%	0.05%	0.19%	0.42%
TLC Origin	(Rf 0)	0.59%	0.65%	0.63%	0.69%	0.16%	0.28%
% Aqueous:		0.76%	0.34%	0.54%	0.36%	0.27%	0.13%
% PES:		0.84%	0.50%	1.39%	1.08%	0.68%	0.43%
Mass Balance:		**4.23%**	**2.00%**	**3.61%**	**2.60%**	**1.73%**	**0.90%**

* Average of 7 Subjects. No Day 2,3 sample available from Subject 4.
BDL = Below Detection Limit.

Eisai America, Inc.
Protocol: E2020-A001-004
Investigator: James Kisicki, M.D.

Table 8
Pharmacokinetic Parameters for E2020 and ^{14}C-E2020 *

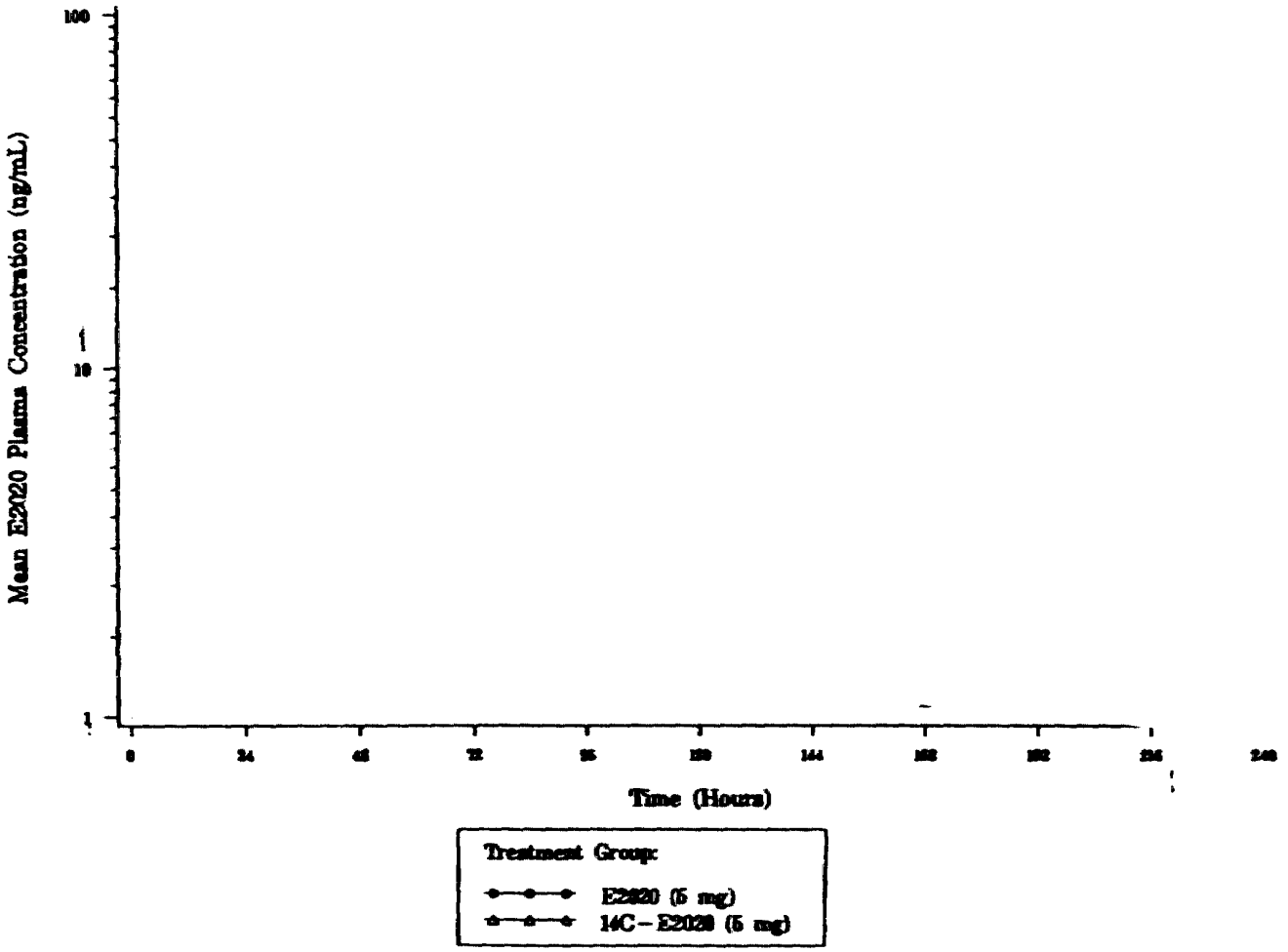
		E2020 (5mg)	^{14}C-E2020 (5mg)
$AUC_{0-144\ hrs.}$ (ng*hr/mL)	N	8	8
	Mean (S.E.)	355.0 (20.7)	663.5 (23.0)
	Min - Max	244.5 - 438.0	588.0 - 768.1
$AUC_{0-\infty}$ (ng*hr/mL)	N	8	8
	Mean (S.E.)	507.4 (31.8)	897.3 (36.1)
	Min - Max	325.2 - 609.9	779.6 - 1066
t_{max} (hour)	N	8	8
	Mean (S.E.)	4.4 (0.5)	4.6 (0.6)
	Min - Max	3.0 - 8.0	3.0 - 8.0
C_{max} (ng/mL)	N	8	8
	Mean (S.E.)	7.2 (0.6)	11.4 (0.8)
	Min - Max	5.5 - 11.1	8.2 - 14.6
λ_z (hour^{-1})	N	8	8
	Mean (S.E.)	0.009 (.001)	0.010 (.001)
	Min - Max	0.007 - 0.014	0.008 - 0.012
$t_{1/2}$ (hour)	N	8	8
	Mean (S.E.)	78.4 (5.5)	70.6 (3.6)
	Min - Max	51.3 - 99.5	56.6 - 85.4
Cl/F (L/hour/kg)	N	8	8
	Mean (S.E.)	0.129 (.009)	0.072 (.003)
	Min - Max	0.104 - 0.181	0.060 - 0.084
$V_{\lambda z}$/F (L/kg)	N	8	8
	Mean (S.E.)	14.25 (.613)	7.250 (.315)
	Min - Max	11.85 - 16.67	6.092 - 8.703
Ratio $AUC_{0-144\ hrs.}$ E2020/Total ^{14}C (%)	N	8	
	Mean (S.E.)	53.7 (3.1)	
	Min - Max	38.9 - 64.6	

Data Source: Appendix VI

* *For ^{14}C-E2020 pharmacokinetic calculations, total radioactivity (dpm) was converted to 'ng E2020 equivalents/ml.'*

Figure III
E2020 – A001 – 004

E2020 and 14C – E2020 Plasma Concentration (ng/mL)



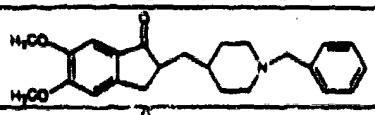
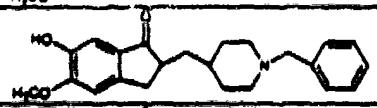
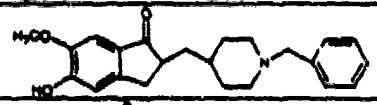
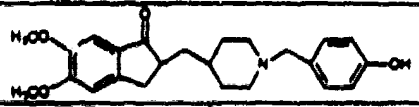
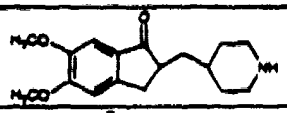
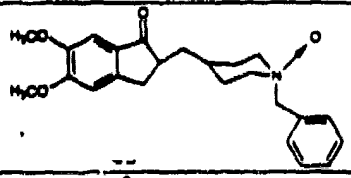
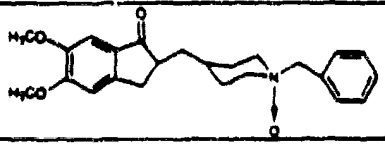
METABOLITES – AchE inhibition

Results

The inhibitory effects of E2020 and six of its putative metabolites on AChE *in vitro* are shown in Table 1. The IC_{50} values for AChE inhibition of E2020, M1, M2, M3, M4, M5, and M6 were 4.9 ± 0.1, 6.0 ± 0.3, 1131 ± 27, 2.4 ± 0.2, 9246 ± 845, 390 ± 4, and 314 ± 7 nM, respectively.

Two metabolites, M1 and M3, among the tested metabolites showed almost the same potency as E2020 in inhibition of AChE *in vitro*. The other metabolites, M2, M4, M5, and M6 were 64 to 1887 times less potent than E2020.

Table 1 Inhibitory effects of E2020 and its putative metabolites on rat brain AChE *in vitro*.

Compound	Chemical Structure	IC_{50} (nM)
E2020		4.9 ± 0.1
M-1		6.0 ± 0.3
M-2		1131 ± 27
M-3		2.4 ± 0.2
M-4		9246 ± 845
M-5		390 ± 4
M-6		314 ± 7

Values represent the mean ± SE from 4 dose - response curves for each test compound.

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METABOLISM - INVITRO STUDY 19952792

Identification of CYP 450 INVOLVED IN THE METABOLISM OF E2020

Summary

E2020, the hydrochloride salt of (R, S)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methylpiperidine, is a novel inhibitor of acetylcholinesterase which is being developed for treatment of the symptoms of Alzheimer's Disease.

Previous studies have demonstrated that human liver microsomes metabolize E2020 mainly by N-dealkylation to M4, and to a lesser extent by O-dealkylation to M1 and M2. In this study, the metabolic profiles of E2020 were investigated in microsomes which expressed specific P450 enzymes. This characterization of the human isozymes involved in the metabolism of E2020 allows the potential for interaction with other drugs to be predicted, Moreover, if the variability of these enzymes in the patient population is known, the extent of in vivo variability in the formation of E2020 metabolites may be estimated.

The results showed that M4, which is the major human metabolite of E2020 in vitro, was formed mainly by CYP3A4 and to a lesser extent by CYP2C9. The formations of M1 and M2 were both mediated mainly by CYP2D6.

From these results, the in vivo metabolic clearance of E2020 is likely to be mediated mainly by CYP3A4 and drug interactions may potentially occur with other CYP3A4 substrates and inhibitors.

Keywords : E2020, P450, CYP2C9, CYP2D6, CYP3A4, Liver, Microsomes, Human, In vitro, Enzyme Kinetics, Metabolism

7. Tables and Figures

Table 1 Kinetic parameters for the net formation of E2020 metabolites in specific human P450 enzyme expressed microsomes.

Metabolite	CLint (μL/min/nmole P450)		
	CYP2C9	CYP2D6	CYP3A4
M1	NC	11.3	ND
M2	ND	3.32	ND
M3	ND	ND	ND
M4	1.60	ND	9.62
M5	ND	ND	NC
M6	ND	ND	NC

CYP2C9 : Samples containing 2 mg/mL of microsomal protein were incubated for 60 min at 37 °C

CYP2D6 : Samples containing 0.4 mg/mL of microsomal protein were incubated for 60 min at 37 °C

CYP3A4 : Samples containing 1 mg/mL of microsomal protein were incubated for 120 min at 37 °C

NC : not calculable, as the formation does not depend on the concentration of E2020 or does not fit Michaelis-Menten kinetics

ND : no difference between expressed and control microsomes

Table 2 Kinetic parameters for the formation of E2020 metabolites, normalized for the specific human P450 content of human microsomes.

Metabolite	CLint (μL/min/100 mg protein)		
	CYP2C9	CYP2D6	CYP3A4
M1	NC	5.65	ND
M2	ND	1.66	ND
M3	ND	ND	ND
M4	9.60	ND	92.4
M5	ND	ND	NC
M6	ND	ND	NC

P450 content in human liver microsomes ; CYP2C : 0.060 nmol/mg protein, CYP2D6 : 0.005 nmol/mg protein, CYP3A : 0.096 nmol/mg protein [4)]

NC : not calculable, as the formation does not depend on the concentration of E2020 or does not fit Michaelis-Menten kinetics

ND : no difference between expressed and control microsomes

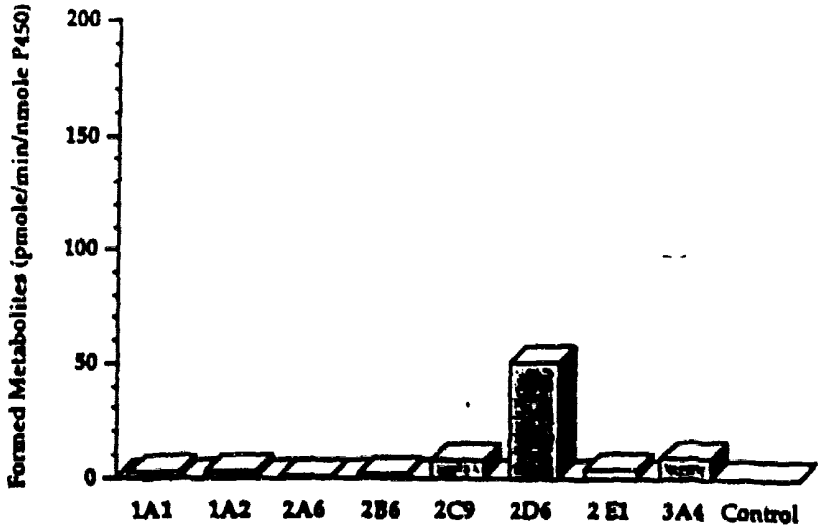

Fig. 3 **The formation of E2020 metabolite M1 in specific human P450 expressed microsomes.**

Mixtures containing 1 mg/mL of microsomal protein and E2020 at 10 μM were incubated for 120 min at 37 °C.

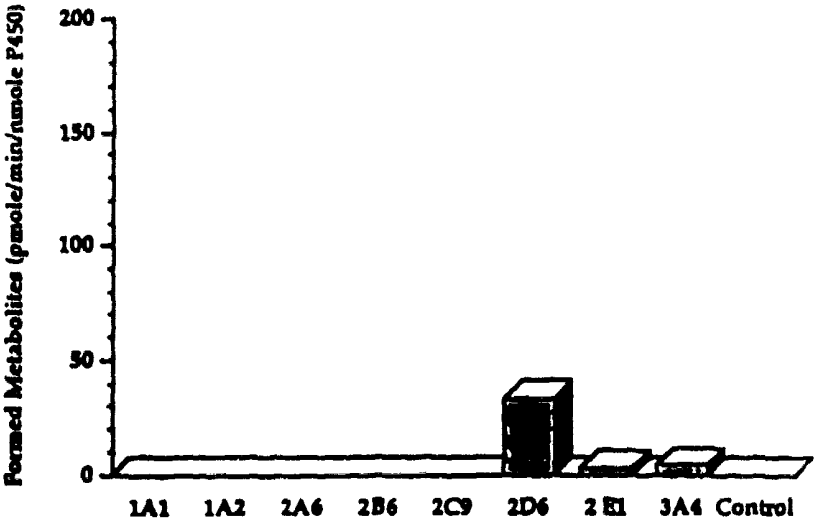

Fig. 4 **The formation of E2020 metabolite M2 in specific human P450 expressed microsomes.**

Mixtures containing 1 mg/mL of microsomal protein and E2020 at 10 μM were incubated for 120 min at 37 °C.

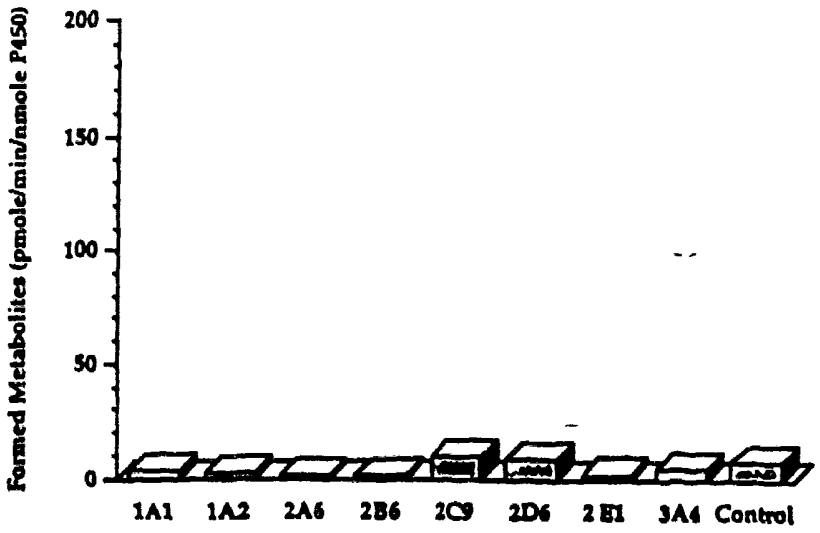

Fig. 5 **The formation of E2020 metabolite M3 in specific human P450 expressed microsomes.**

Mixtures containing 1 mg/mL of microsomal protein and E2020 at 10 μM were incubated for 120 min at 37 °C.

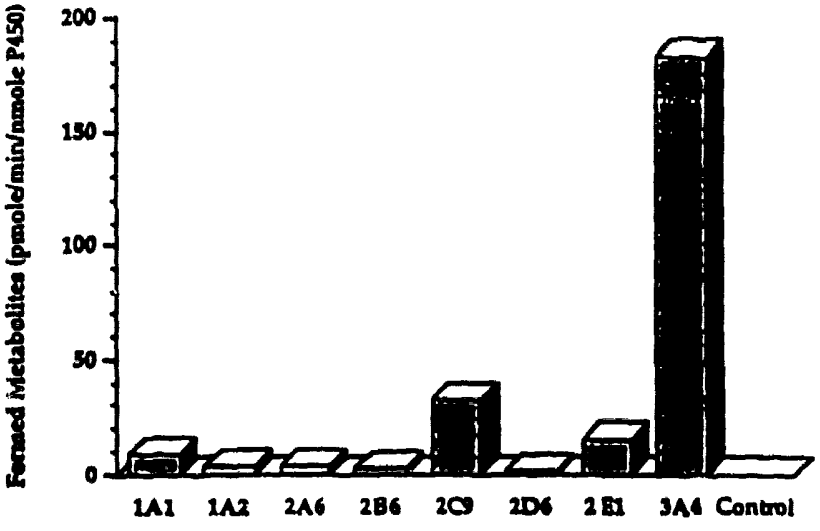

Fig. 6 **The formation of E2020 metabolite M4 in specific human P450 expressed microsomes.**

Mixtures containing 1 mg/mL of microsomal protein and E2020 at 10 μM were incubated for 120 min at 37 °C.

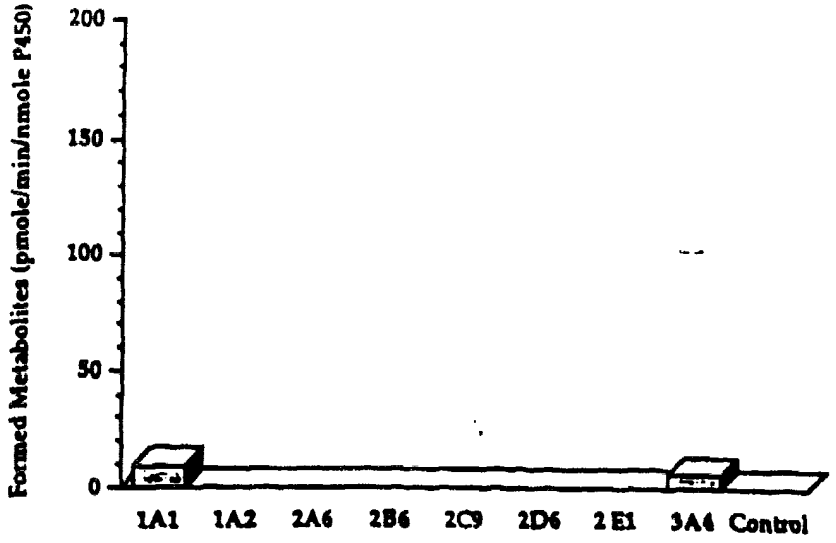

Fig. 7 **The formation of E2020 metabolite M5 in specific human P450 expressed microsomes.**
Mixtures containing 1 mg/mL of microsomal protein and E2020 at 10 μM were incubated for 120 min at 37 °C.

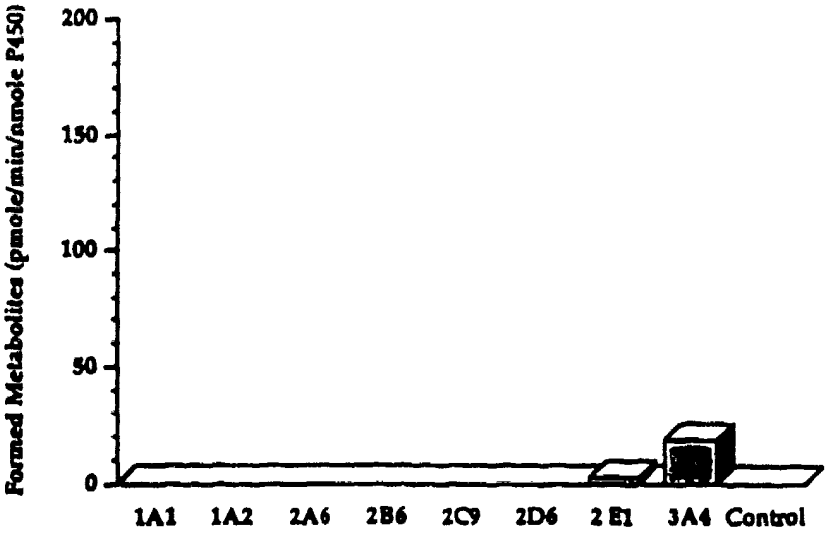

Fig. 8 **The formation of E2020 metabolite M6 in specific human P450 expressed microsomes.**
Mixtures containing 1 mg/mL of microsomal protein and E2020 at 10 μM were incubated for 120 min at 37 °C.

STUDY D23
METABOLISM - IN VITRO

Interaction of E2020 with Human Cytochromes P450

Summary

IC50 values for E2020 were determined against isoform-selective substrates of cytochrome P450 1A2, 2C9, 2C19, 2D6, and 3A4 and were all greater than 100 µM. Ki values for the interaction of E2020 with CYP3A4 and CYP2D6 were also determined. Mean Ki values for three individual sets of human liver microsomes were 131 and 47 µM for CYP3A4 and CYP2D6, respectively. Both interactions were typical of competitive inhibition. Steady-state Cmax concentrations of E2020, at an anticipated therapeutic daily dose of 10 mg, would be approximately 62 ng/ml = 164 nM. This concentration was calculated from the steady-state Cmax (31 ng/ml) achieved during the multiple dose study at a dose of 5 mg (Study no. A001-002; Harris Laboratories) and assuming dose-proportional exposure. Since E2020 anticipated therapeutic concentrations are more than 280-fold lower than the lowest Ki value obtained with CYP2D6 and almost 800-fold lower than the Ki observed with CYP3A4, drug-drug interactions as a consequence of administration of E2020 are not anticipated.

Objective

To determine the interaction of E-2020 with human liver microsomal cytochromes P450 in order to predict the potential for drug-drug interactions *in vivo*.

Methods

Stocks of human liver microsomes are maintained at -70° C. Microsomes were prepared from frozen human liver tissue by standard methods (Appendix 1). Details of the human livers are included in Appendix 2. Cytochrome P450 (CYP) concentrations were determined by the method of Omura and Sato and protein concentrations by the standard BCA procedure (Smith e The activities of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 were assayed by standard procedures using the probe substrates phenacetin, tolbutamide, S-mephenytoin, bufuralol and testosterone, respectively. Methods are detailed in Appendix 3.

IC50 and Ki values were determined using human liver microsomal preparations from 3 different individuals designated HL 1016, HL 1018 and HL 1020. CYP concentrations were 0.1 µM for phenacetin O-deethylase, bufuralol 1'-hydroxylase and testosterone 6β-hydroxylase assays, and 0.5 µM for S-mephenytoin hydroxylase and tolbutamide hydroxylase assays. All microsomal reactions were terminated at 20 minutes.

To evaluate whether significant metabolism of E2020 was occurring in the experiments to determine IC50 values, curves for the interaction of E2020 with CYP2D6 and with CYP2C19 were generated in the presence of 1 µM ketoconazole, a concentration of ketoconazole which selectively inhibits CYP3A4. IC50 values were compared to those generated in the absence of ketoconazole and were similar indicating that E-2020 concentrations were not being significantly depleted during the course of the incubation. Additional studies were performed in the absence of ketoconazole.

IC50 values were generated for E-2020 inhibition of CYP1A2, 2C9, 2C19, 2D6 and 3A4-mediated metabolism of the isoform specific substrates phenacetin (10 μM), tolbutamide (200 μM), S-mephenytoin (50 μM), bufuralol (10 μM) and testosterone (20 μM), respectively. E-2020 concentrations used were 0, 0.09, 0.27, 0.91, 2.7, 9.1, 27, and 91 μM. IC50 curve fitting was performed using the program DeltaGraph Version 2.0 for Windows (DeltaPoint Inc.) with the equation $y=100-(100\frac{x}{x+B})$, where y=metabolic rate, x=inhibitor concentration, and B=IC50 (convergence<0.1). To ensure that chemical stability of E2020 in methanol was not an issue, stock solutions were prepared on each assay day. Details of stock solution preparation can be found in Appendix 4.

The IC50 values generated were used to select suitable concentrations at which to produce Dixon plots (1/rate of metabolism vs inhibitor concentration) for determination of Ki values for CYP2D6 and CYP3A4. To calculate Ki values for CYP2D6, concentrations of bufuralol were 5, 10 and 20 μM and concentrations of E-2020 were 0, 73, 109, and 182 μM. To determine Ki values for CYP3A4, testosterone concentrations were 20, 40 and 80 μM and E-2020 concentrations were 0, 73, 109, and 182 μM. Ki values for each set of human liver microsomes were calculated from Dixon plots as mean values of the intercepts for each pair of substrate concentration lines. Linear regressions were calculated using a least squares fit using the program DeltaGraph Version 2.0 for Windows.

Results

The interaction of E-2020 with each of the five major drug-metabolizing human isoforms of CYP was investigated by measuring the inhibition of probe substrate activities by E-2020, illustrated in Figure 1 and summarized as IC50 values in Table 1. IC50 values were greater than 100 μM for E-2020 inhibition of all five probe substrates. IC50 values were extrapolated for S-mephenytoin hydroxylase, bufuralol 1'-hydroxylase and testosterone 6β-hydroxylase activities and were approximately 213 μM, 108 μM and 152 μM, respectively. There was little or no inhibition at 100 μM E-2020 concentrations for the remaining two activities (phenacetin O-deethylase and tolbutamide hydroxylase activities).

A comparison of IC50 values in the presence and absence of 1 μM ketoconazole, which inhibits CYP3A4, demonstrated that there was no significant depletion of E2020 by this isoform during these incubations. In the presence and absence of ketoconazole, IC50 values for the interaction of E2020 with CYP2D6 using human liver microsomes HL1020, were 107 μM and 122 μM, respectively, and for the interaction of E2020 with CYP2C19, using human liver microsomes HL 1016, were 135 μM and 153 μM, respectively. As the IC50 values were similar, only the data for the interaction of E2020 with CYP2D6 in the presence of ketoconazole and the interaction of E2020 with CYP2C19 in the absence of ketoconazole are shown in Table 1 and illustrated in Figure 1.

E-2020 interactions with CYP2D6 (bufuralol 1'-hydroxylase) and CYP3A4 (testosterone 6β-hydroxylase) were further profiled by determining Ki values. Ki values were obtained for E-2020 using 3 probe substrate concentrations and 4 inhibitor concentrations as illustrated in Dixon plots (Figures 2 and 3) and summarized in Table 2. Mean Ki values obtained were

47 μM for CYP2D6 and 131 μM for CYP3A4. Both interactions were typical of competitive inhibition.

A summary of the data used to generate the IC50 and Ki graphs is included in Appendix 5.

Discussion

The interaction of E2020 with each of the five major drug-metabolizing isoforms of cytochrome P450 has been investigated by determining the ability of E2020 to inhibit the metabolism of isoform-selective substrates of these isoforms. IC50 values for the interaction of E2020 with each of the CYP isoforms were all greater than 100 μM. Ki values were also determined for the interaction of E2020 with two of these isoforms, CYP2D6 and CYP3A4, and were 47 and 131 μM, respectively. Steady-state Cmax concentrations of E2020, at an anticipated therapeutic daily dose of 10 mg, would be approximately 62 ng/ml = 164 nM. This concentration was calculated from the steady-state Cmax (31 ng/ml) achieved during the multiple dose study at a dose of 5 mg (Study no. A001-002; Harris Laboratories) and assuming dose-proportional exposure. Since E2020 anticipated therapeutic concentrations are more than 280-fold lower than the lowest Ki value obtained with CYP2D6 and almost 800-fold lower than the Ki observed with CYP3A4, drug-drug interactions as a consequence of administration of E2020 are not anticipated.

References

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Smith, P.K. *et al.* (1985) Analyt. Biochem. 150, 76-85.

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TABLE 1
IC50 VALUES FOR E-2020 INTERACTION WITH HUMAN LIVER MICROSOMAL CYP1A2, CYP2C9, CYP2C19, CYP2D6 AND CYP3A4

HUMAN LIVER MICROSOMES	CYP1A2[a]	CYP2C9[b]	CYP2C19[c]	CYP2D6[d]	CYP3A4[e]
HL 1016	>>100	>>100	153	99	180
HL 1018	>>100	>>100	147	117	112
HL 1020	>>100	>>100	339	107	165
MEAN ± S.D.	>> 100 μM	>> 100 μM	213 μM ± 109	108 μM ± 9	152 μM ± 36

[a] CYP1A2 activity determined by phenacetin O-deethylation.
[b] CYP2C9 activity determined by tolbutamide hydroxylation.
[c] CYP2C19 activity determined by S(+)mephenytoin hydroxylation.
[d] CYP2D6 activity determined by bufuralol 1'-hydroxylation.
[e] CYP3A4 activity determined by testosterone 6β-hydroxylation.

TABLE 2
Ki VALUES (μM) FOR E-2020 INTERACTION WITH HUMAN LIVER MICROSOMAL CYP2D6 AND CYP3A4

HUMAN LIVER MICROSOMES	Ki (μM)	
	CYP2D6[a]	CYP3A4[b]
HL 1016	27	150
HL 1018	53	104
HL 1020	62	140
MEAN ± S.D.	47 ± 18	131 ± 24

[a] CYP2D6 activity determined by bufuralol 1'-hydroxylase activity.

[b] CYP3A4 activity determined by testosterone 6β-hydroxylase activity.

STUDY 19952805 (METABOLISM - IN VIT.

IN VITRO E2020 Drug Interaction Study in Human Liver Microsomes.

Summary

E2020, the hydrochloride salt of (R, S)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methylpiperidine, is a novel inhibitor of acetylcholinesterase which is being developed for treatment of the symptoms of Alzheimer's Disease.

In this study, to confirm the involvement of CYP3A4 and CYP2D6 in the metabolism of E2020, the inhibitory effects of potent inhibitors of CYP3A4 (ketoconazole) and CYP2D6 (quinidine) on the metabolism of E2020 were studied in human liver microsomes.

The formation of M4, the main metabolite of E2020 in human microsomes, was inhibited by >90 % by 2 μM of ketoconazole, which is the potent inhibitor of CYP3A4. Quinidine, which is a substrate of CYP3A4, had little inhibitory effect on the M4 formation at the low concentration of 1 μM. However, at the higher concentration of 100 μM, the formation of M4 was inhibited by about 45 %. Moreover, the inhibitory effect of the combination of the two drugs, was equal to that of ketoconazole alone. These results showed that the formation of M4 is mainly mediated by CYP3A4, and the contribution of other P450 isoenzymes appears to be low.

The formations of M1 and M2 were inhibited by 77.0 % and 58.4 %, respectively, by 100 μM of quinidine, which is a potent inhibitor of CYP2D6. The formations of M1 and M2 were also inhibited by ketoconazole, by about 20 % and 30 %, respectively, at the highest concentration of 2 μM. The combination of ketoconazole (2 μM) and quinidine (100 μM) markedly inhibited the formation of M1 and M2, by 85.1 and 74.2 %, respectively. These results showed that M1 and M2 are mainly formed by CYP2D6.

In conclusion : this study confirmed that the metabolism of E2020 is mainly mediated by CYP3A4 and to a lesser extent by CYP2D6, and little contribution is expected from the other P450 isoenzymes.

Keywords : E2020, Human liver microsomes, In vitro, Metabolism, Inhibition, Quinidine, Ketoconazole

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4. Results

The effect of ketoconazole and quinidine on the metabolism of E2020 in human liver microsomes was studied (Table 1 and Fig. 3 to 5). The individual data are tabulated in Appendix 4.

Ketoconazole is a very potent inhibitor of the CYP3A4 [3] and slightly inhibits CYP1A2, CYP2C9 and CYP2D6 mediated reactions [6]. Quinidine selectively inhibits CYP2D6 mediated reactions [4] and is also a substrate of CYP3A4 [6].

4.1 The Effect of Inhibitors on the Formation of M1

As shown in Table 1 and Fig. 3, ketoconazole slightly inhibited the formation of M1, by 12.3, 18.7 and 21.7 % at the concentrations of 0.2, 1 and 2 μM. On the other hand, quinidine inhibited the formation of M1 by 58.0, 66.7 and 77.0 % at the concentrations of 1, 10 and 100 μM, respectively. The inhibitory effects of the combination were slightly higher than the effect of quinidine alone, with 85.1 % inhibition of the formation of M1 at the concentrations of 2 μM of ketoconazole and 100 μM quinidine.

4.2 The Effect of Inhibitors on the Formation of M2

As shown in Table 1 and Fig. 4, ketoconazole slightly inhibited the formation of M2, by 14.0, 26.8 and 33.0 % at the concentrations of 0.2, 1 and 2 μM. On the other hand, quinidine inhibited the formation of M1 by 38.0, 49.6 and 58.4 % at the concentrations of 1, 10 and 100 μM, respectively. The inhibitory effects of the combination were slightly higher than the effect of quinidine alone, with 74.2 % inhibition of the formation of M2 at the concentrations of 2 μM of ketoconazole and 100 μM quinidine.

4.3 The Effect of Inhibitors on the Formation of M4

As shown in Table 1 and Fig. 5, ketoconazole markedly inhibited the formation of M4, by 61.5, 88.2 and 91.5 % at the concentrations of 0.2, 1 and 2 μM, and quinidine slightly inhibited the formation of M4 by 3.70, 12.9 and 45.2 % at the concentrations of 1, 10 and 100 μM, respectively. The inhibitory effects of the combination were similar to the effects of ketoconazole alone, with 87.1 % inhibition of the formation of M4 at the concentrations of 2 μM of ketoconazole and 100 μM quinidine.

7. Tables and Figures

Table 1 **Inhibition by ketoconazole and quinidine of E2020 metabolism in human liver microsomes.**

	Formed Metabolites (% of control)			Inhibitory Effects (%)		
	M1	M2	M4	M1	M2	M4
Control	100	100	100	-	-	-
ketoconazole 0.2μM	87.7	86.0	38.5	12.3	14.0	61.5
ketoconazole 1μM	81.3	73.2	11.8	18.7	26.8	88.2
ketoconazole 2μM	78.3	67.0	8.48	21.7	33.0	91.5
quinidine 1μM	62.0	62.0	96.3	38.0	38.0	3.70
quinidine 10μM	33.3	50.4	87.1	66.7	49.6	12.9
quinidine 100μM	23.0	41.6	54.8	77.0	58.4	45.2
ketoconazole 0.2μM + quinidine 10μM	24.2	40.2	41.2	75.8	59.8	58.8
ketoconazole 1μM + quinidine 1μM	25.6	38.7	19.2	74.4	61.3	80.8
ketoconazole 2μM + quinidine 100μM	14.9	25.8	12.9	85.1	74.2	87.1

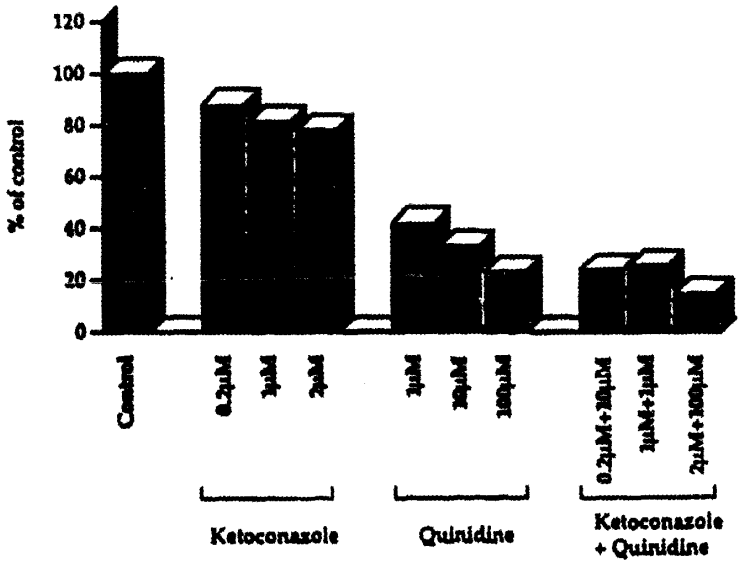

Fig. 3 **Inhibitory effects of ketoconazole and quinidine on the formation of E2020 metabolite M1.**
Mixtures containing an E2020 concentration of 50 μM and 1 mg/mL of microsomal protein were incubated for 5 min at 37 °C.

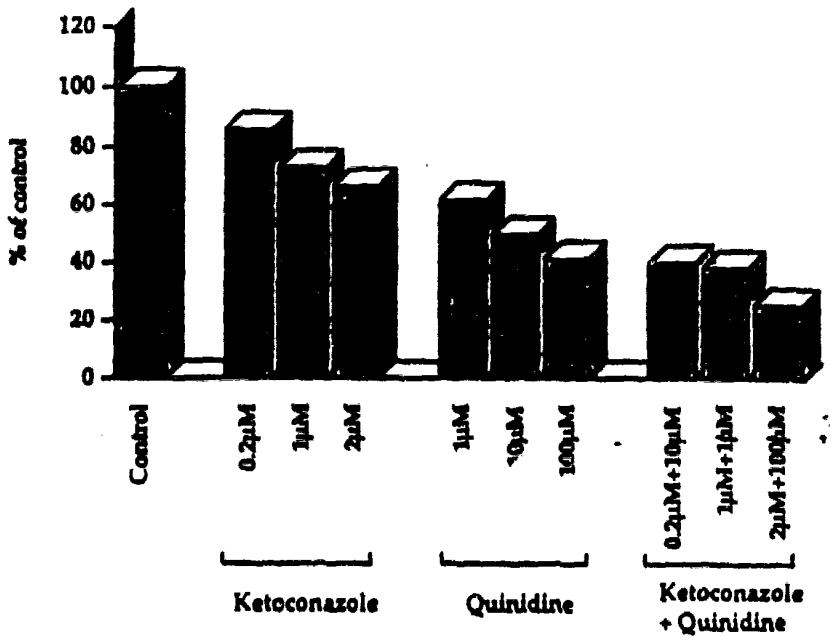

Fig. 4 **Inhibitory effect of ketoconazole and quinidine on the formation of E2020 metabolite M2.**

Mixtures containing an E2020 concentration of 50 μM and 1 mg/mL of microsomal protein were incubated for 5 min at 37 °C.

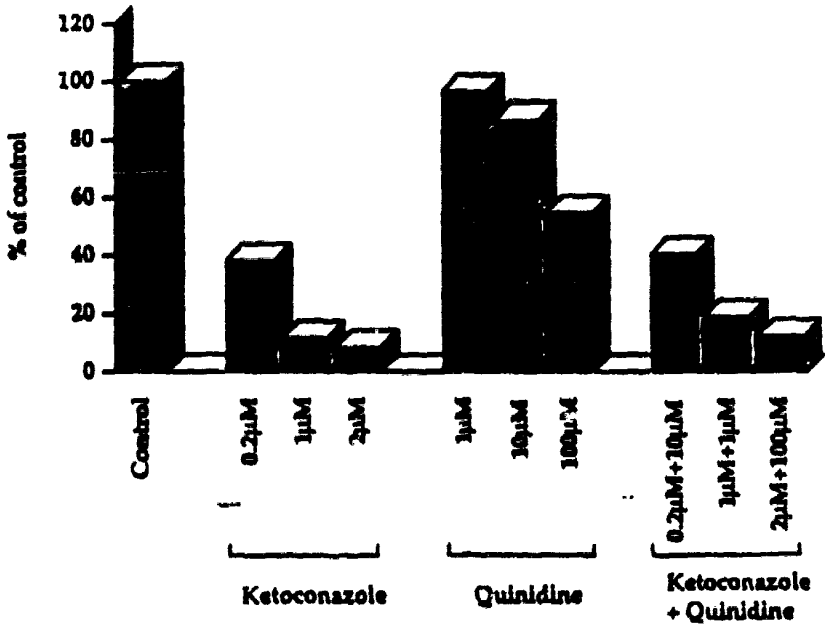

Fig. 5 **Inhibitory effect of ketoconazole and quinidine on the formation of E2020 metabolite M4.**

Mixtures containing an E2020 concentration of 50 μM and 1 mg/mL of microsomal protein were incubated for 5 min at 37 °C.

MULTIPLE DOSE STUDY - CSF/PLA MA

CLINICAL STUDY SYNOPSIS

Title: A Single and Multiple-Dose Study of the Central and Peripheral Pharmacokinetics and Pharmacodynamics of E2020, An Acetylcholinesterase Inhibitor, in Healthy Volunteers.
Study #: E2020-A001-003

Investigator:

Objective: The primary objective of this study was to establish central (cerebrospinal fluid) and peripheral (venous blood) pharmacokinetic (PK) and pharmacodynamic (PD) profiles of E2020 following administration of a single 5-mg oral dose, and again at the time of steady-state, following multiple-dose administration of either 5 or 10-mg single daily doses. The secondary objective of this study was to assess the acute and then chronic effects of E2020 on four neuroendocrine measures, corticotropin releasing factor (CRF), adrenocorticotropic hormone (ACTH), vasopressin and cortisol, at specified times during the study.

Study Design: This was a double-blind, randomized, single and multiple-dose study.

Study Population: Volunteers for this study were healthy, ambulatory, non-smoking male and female volunteers greater then 18 years of age. A total of 14 volunteers were randomized into this study and 12 completed the study. Participants were Caucasion (12), Black (1) and Asian (1). The 5-mg group was comprised of 5 males and 2 females and ranged in age from 20 to 52 years and in weight from 64.5 to 80.9 kg. The 10-mg group was comprised of 3 males and 4 females and ranged in age from 27 to 64 years and in weight from 64.1 to 87.3 kg.

Treatment Groups: Study subjects were randomized to receive either 5-mg or 10-mg daily doses of E2020 for 28 days. Prior to initiation of the multiple-dose phase, however, all study subjects received a single 5-mg dose of E2020 to allow for characterization of central and peripheral E2020 pharmacokinetics following single-dose administration. All subjects received the 5-mg dose during the single-dose phase in order to avoid a reaction to the acute, extensive inhibition of cholinesterase which has been observed when the 10-mg dose is administered to naive subjects. In addition, during the 28-day multiple-dose phase, subjects randomized to the 10-mg group initially received 5-mg daily doses of E2020 for 7 days before receiving their first 10-mg dose.

Methods *(Clinical)*: After providing written informed consent, volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to randomization into the study. Subjects were admitted to the study site in the evening, 48 hours prior to the 5-mg single-dose phase of drug administration. Following admission, a physical examination and a urine drug screen were conducted. During the following day, an indwelling lumbar intrathecal catheter was placed for the collection of CSF. CSF was collected at a rate of 6-ml per hour beginning 25 hours prior to drug administration and continuing for 24 hours after drug administration. Venous blood samples were drawn concurrently at specified intervals during the 48-hours of CSF collection, and continued up to 120 hours post-drug administration. Subjects remained at the study site for a minimum of 24 hours following withdrawal of the intrathecal catheter. Following a physical and laboratory evaluation, subjects were discharged from the study site.

There was a 7-day drug-free interval between discharge from the single-dose phase and the start of the 28-day multiple-dose phase. During the multiple-dose phase, subjects took their E2020 doses on an outpatient basis. Following initiation of the multiple-dose phase, subjects had vital signs assessed and provided venous blood samples for pharmacokinetic and pharmacodynamic measures at specified times during the 28-day period. On study days 17, 24 and 31, routine physical examinations and medication compliance checks were conducted. Monitoring for adverse experiences was done continuously.

Subjects were readmitted to the study site 24 hours prior to final dose administration. In contrast to the single-dose phase, CSF and venous blood sampling began 1 hour prior to drug administration, and continued for 48 hours after drug administration. Placement and removal of the CSF catheter was conducted accordingly. Prior to discharge, a routine physical examination was performed. At specified times during the post-drug phase (to 240 hours), subjects provided additional blood samples for PK/PD analysis. Ten days after the final dose of E2020 was administered, subjects returned to the study site for a final physical and laboratory evaluation.

Methods *(Analytical)*: E2020 concentrations in plasma were determined by . E2020 concentrations in CSF were measured using a method. Acetylcholinesterase activity in red blood cell membranes (rbc-AChE) and in CSF was assessed using specific radioenzyme assays. The measurement of endocrine levels in plasma and CSF was done at using standard methodology.

E2020 PK and PD were characterized using the resultant analytical data and standard techniques. Following the doses administered on

E2020 Plasma Concentration Parameters at Steady State (Geometric Means)

	C_{min} (ng/ml)	C_{max} (ng/ml)	C_{ss} (ng/ml)	$AUC_{(0-24)}$ (ng*hr/ml)
5-mg dose	13.9	27.1	19.1	459.2
10-mg dose	36.1	66.1	46.6	1118.8

Pharmacokinetics - *Cerebrospinal Fluid (CSF):* The quantification of E2020 in CSF was compromised in this study by adsorption of E2020 to the sample collection tubing, precluding the collection of complete profiles in all but two subjects. However, steady-state trough CSF samples drawn directly from the intrathecal catheter by syringe just prior to the last dose of E2020, bypassed the collection tubing and were not compromised by adsorption. A summary table of the E2020 concentrations in CSF and their relationship to corresponding plasma concentrations is presented below. The average CSF : plasma ratio for both doses, expressed as a percent of the concentration in plasma, was 15.7%.

Corresponding Plasma and CSF E2020 Concentrations at Steady-State

	Plasma (ng/ml) Mean (± SD) Min - Max	CSF (ng/ml) Mean (± SD) Min - Max	Ratio (%) Mean (± SD) Min - Max
5-mg dose	16.5 (2.8) 12.5 - 20.9	3.0 (1.2) 1.8 - 5.2	18.4 (7.1) 11.0 - 29.7
10-mg dose	43.9 (21.3) 26.0 - 73.4	5.8 (3.2) 3.1 - 9.9	13.0 (2.4) 9.1 - 16.3

Pharmacodynamics - *Plasma:* A significant relationshship was observed between E2020 plasma concentration and the percent inhibition of red blood cell peripheral AChE activity (% rbc-AChEI). This relationship was consistent during both of the 24-hour periods evaluated (Days 1 and 38) and demonstrates that there are no time-dependent changes in E2020 pharmacodynamics. The average percent AChEI for the 24-hour period measured on Day 1 (single-dose) was 20.6%. On Day 38 (steady-state), the average percent AChEI was 55.9% for the 5-mg dose group and 72.3% for the 10-mg dose group.

PHARMACOKINETICS: CEREBROSPINAL FLUID

5 mg	1			
	4			
	6			
	7			
	11			
	21			
Mean				
SD				
10 mg	2			
	3			
	5			
	10			
	12			
	20			
Mean				
SD				

TABLE 11: Distribution of E2020 into CSF: Trough Plasma and CSF Concentration Ratios at Steady-State.

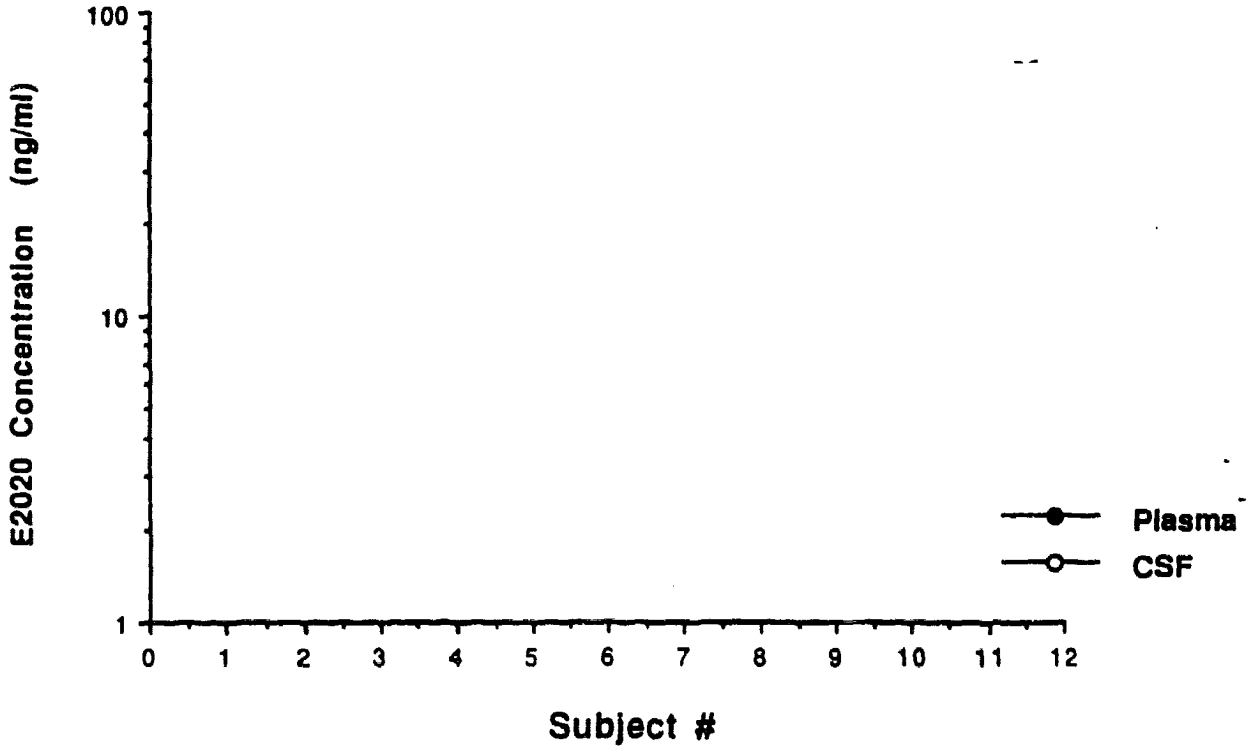

Figure IV: Corresponding plasma and CSF values for each subject at steady-state. All samples were drawn prior to the last E2020 dose on study Day 38.

Note: Subject 008 = Subject 020 and Subject 009 = Subject 021.

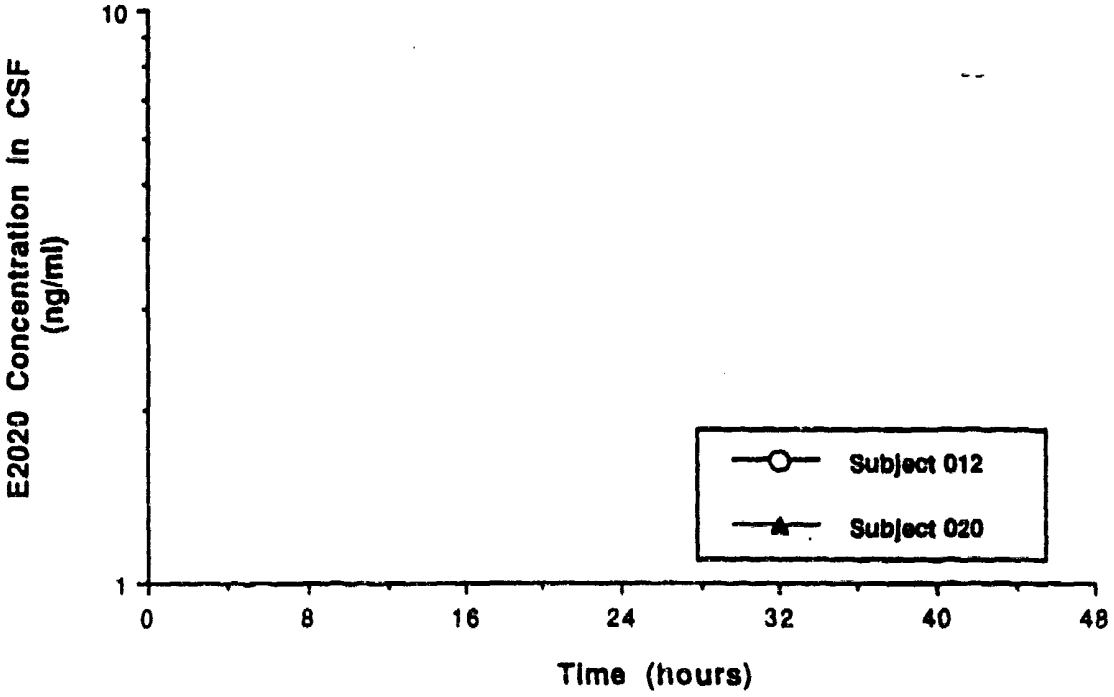

Figure V: E2020 concentration versus time profile in CSF following dose-administration (10-mg) at steady-state (Day 38). E2020 concentrations were determined by an LC/MS/MS method.

Hepatic Study

CLINICAL STUDY SYNOPSIS

Title: A Single-dose Study of the Pharmacokinetics of E2020, An Acetylcholinesterase Inhibitor, in Patients with Hepatic Disease. Study #: E2020-A001-005

Investigator:

Objective: The primary objective of this study was to assess the effect of compromised hepatic function on the pharmacokinetics of E2020. This was done by comparing the pharmacokinetics of E2020 in subjects with stable hepatic disease (alcoholic cirrhosis) to those in healthy age- and sex-matched controls, following administration of a single 5-mg oral dose of E2020.

Study Design: This was a single-dose, non-randomized, open-label study which was conducted at a single investigational site.

Study Population: Ten patients with stable alcoholic cirrhosis and ten healthy, age and sex-matched volunteers were enrolled into this study. All twenty participants completed the study without incident. Patient volunteers were Caucasian (8) and Black (2) and ranged in age from 39 to 58 years (mean ± SD: 46.7 ± 2.0 years). Healthy volunteers were Caucasian (5) and Black (5) and ranged in age from 36 to 61 years (mean ± SD: 46.5 ± 2.6 years). All study participants were males.

Treatment Groups: All study participants received a single, 5-mg oral dose of E2020. Medication was administered after a fast of at least eight hours and was accompanied by 250 ml of tap water.

Methods *(Clinical)*: After providing written informed consent, volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to the start of the study. Appropriate subjects were admitted to the study site on the evening prior to drug administration. Upon admission, a routine physical examination was conducted. Entry vital signs (sitting position), and weight were recorded, and urine specimens for drug use/abuse screening were collected. The following morning, prior to drug administration and following an 8-hour fast, blood samples for laboratory and analytical determinations were collected. Participants were then administered a single 5-mg tablet of E2020 with 250 ml of tap water. All study participants remained at the

study site for 48-hours following drug administration. During this time, blood samples were collected at specified intervals for the measurement of E2020 in plasma. Subjects were discharged from the study site on the morning of Day 3, after providing a 48-hour blood sample. Volunteers then returned to the study site at 24-hour intervals to 120 hours, for additional analytical sample collection.

Methods *(Analytical)*: Concentrations of E2020 in plasma were determined by a method (range of quantitation: 1 - 100 ng/ml). Characterization of E2020 pharmacokinetics was conducted on the resultant analytical data using standard techniques. Parameters used for between-group comparisons included C_{max}, t_{max}, AUC_{0-168}, $AUC_{0-\infty}$, Cl_f and $t_{1/2}$.

Results: Pharmacokinetics: Mean plasma concentration vs time profile is shown in Figure 1. The primary pharmacokinetic parameters for this study are summarized Table 1. No statistically significant differences in E2020 pharmacokinetics were observed between the hepatic patients and the normal subjects with the exception of Cmax and Tamx. The hepatic group showed a statistically higher mean Cmax value of 6.6 ng/ml as compared to the control group which had a mean Cmax value of 4.8 ng/ml. This was an increase in Cmax of 37.5%. The hepatic group achieved peak plasma concentrations faster than the normal subjects, i.e., 3.0 vs 4.5 hrs; a decrease of 33%. The AUC values showed much smaller increases and those differences were not statistically significant. The AUC_{0-168} was found to increase by 7%, and the $AUC_{0-\infty}$ was found to increase by 21%. The $t_{1/2}$ increased by about 20%, while clearance decreased by about 14%, but these differences were found not to be statistically significant.

Conclusions: The results of this study suggest that compromised hepatic function will produce an increase in E2020 plasma concentrations.

Figure 11
E2020 – A001 – 005

E2020 Plasma Concentration (ng/mL)

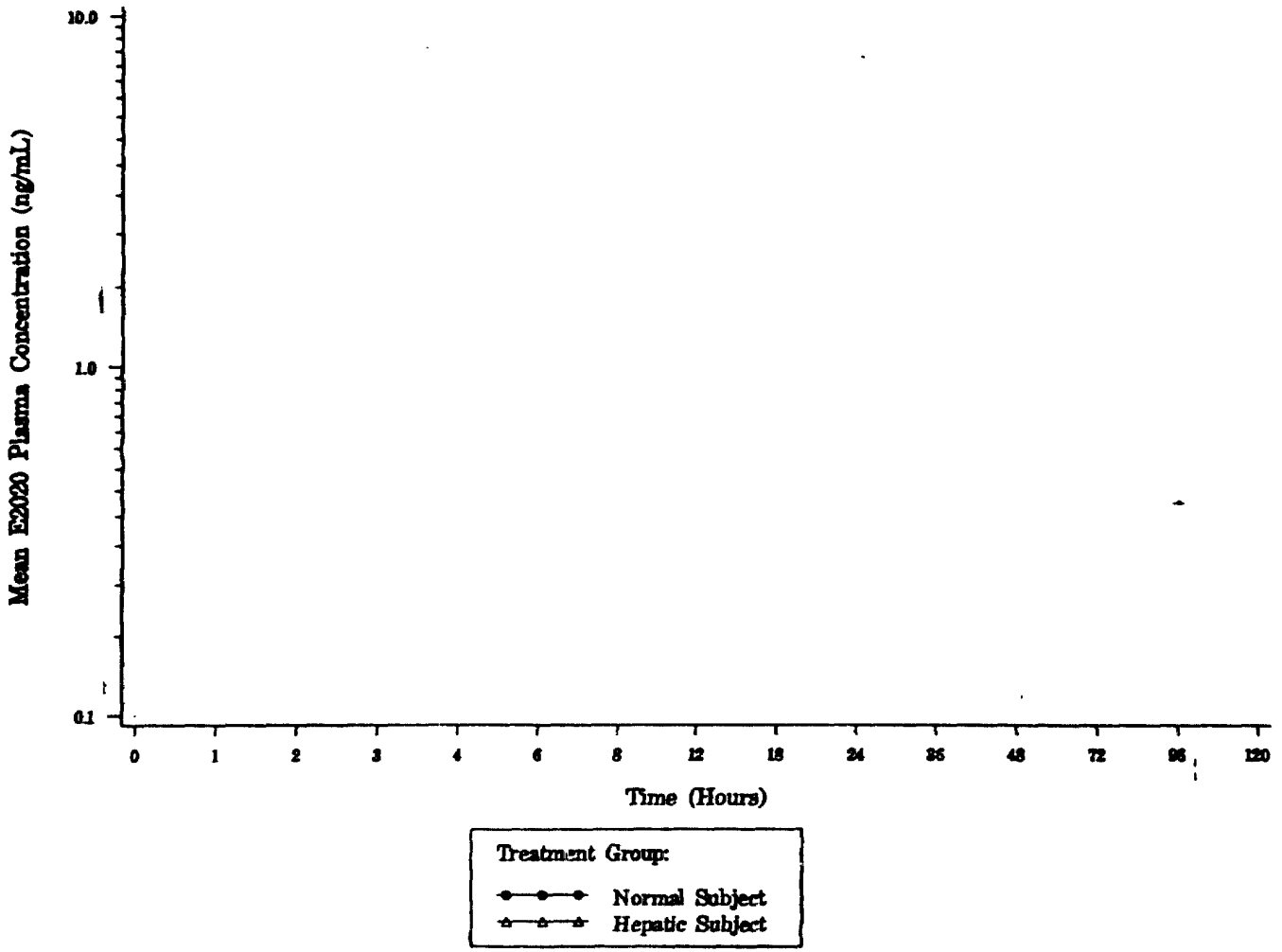

Table 8
E2020 Pharmacokinetic Parameters

		Hepatic Subjects	Normal Subjects	P-value	Estimate and (95% C.I.)*
$AUC_{0\text{-}120\ hrs}$ (ng*hr/mL)					
	N	9	10		
	Mean (S.E.)	247.0 (21.5)	231.7 (27.8)	0.6737	15.304 (-60.05 , 90.65)
	Min - Max	186.5 - 376.6	121.7 - 373.1		
$AUC_{0-\infty}$ (ng*hr/mL)					
	N	9	10		
	Mean (S.E.)	465.0 (67.7)	382.9 (40.5)	0.3019	82.026 (-80.52 , 244.57)
	Min - Max	301.1 - 893.4	193.2 - 658.0		
t_{max} (hour)					
	N	9	10		
	Mean (S.E.)	3.0 (0.7)	4.5 (0.5)	0.0964	-1.500 (-3.30 , 0.30)
	Min - Max	1.0 - 8.0	2.0 - 6.0		
C_{max} (ng/mL)					
	N	9	10		
	Mean (S.E.)	6.6 (0.6)	4.8 (0.6)	0.0218	1.865 (0.31 , 3.42)
	Min - Max	4.1 - 9.3	2.5 - 6.7		
λ_z ($hour^{-1}$)					
	N	9	10		
	Mean (S.E.)	0.010 (.001)	0.011 (.001)	0.5579	-0.001 (-0.01 , 0.00)
	Mini- Max	0.003 - 0.018	0.006 - 0.018		

Data Source: Statistical Appendix VII

* The difference in the means between the two groups and the 95% confidence interval.

Table 8
E2020 Pharmacokinetic Parameters

		Hepatic Subjects	Normal Subjects	P-value	Estimate and (95% C.I)*
$t_{1/2}$ (hour)					
	N	9	10		
	Mean (S.E.)	89.6 (18.1)	71.6 (7.7)	0.3554	18.013 (-21.99 , 58.01)
	Min - Max	38.6 - 223.2	38.4 - 113.7		
Cl_T/F (L/hour/kg)					
	N	9	10		
	Mean (S.E.)	0.158 (.021)	0.184 (.022)	0.4005	-0.027 (-0.09 , 0.04)
	Min - Max	0.054 - 0.233	0.091 - 0.339		
$V_{\lambda 2}/F$ (L/kg)					
	N	9	10		
	Mean (S.E.)	16.67 (1.14)	17.26 (1.13)	0.7172	-0.593 (-3.99 , 2.80)
	Min - Max	11.79 - 21.94	12.92 - 24.62		

Data Source: Statistical Appendix VII

* The difference in the means between the two groups and the 95% confidence interval.

Statistical Appendix VII
Pharmacokinetic Parameters by Subject

GROUP	SUBJECT NUMBER	WEIGHT (KG.)	Cmax (ng/mL)	tmax (hrs.)	AUC(0-120) (ng hr/mL)	AUC(0-inf.) (ng hr/mL)	CL/F (L/hr/kg)	HALF LIFE (hrs.)	TERMINATION RATE CONSTANT(K) (hour^-1)	V/F (L/kg)
(HEPATIC)	001									
	002									
	003									
	004									
	005									
	007									
	008									
	009									
	010									
(NORMAL)	021									
	022									
	023									
	024									
	025									
	026									
	027									
	028									
	029									
	030									

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Renal Study

SUMMARY / SYNOPSIS

This is an interim report prepared on the request of the sponsoring company after 4 subjects with impaired renal function and 4 healthy control subjects had completed the study.

Objectives: To compare the pharmacokinetics of a single dose of E2020 and its major metabolites (M-1 to M-6 and their conjugates) in subjects with moderate -to-severe impaired renal function with a group of age and sex matched healthy control subjects. To assess the safety and tolerance of a single 5mg dose of E2020 in subjects with clinically significant renal disease.

Trial Design and Methods: This was an open, single dose, two centre study in 11 renal patients with creatinine clearances less than 30 $mL \cdot min^{-1}$ / 1.73 m^2 body surface area and 11 aged and sex matched controls. Each volunteer received one 5 mg dose of E2020.

Serial blood samples and urine were collected over 384 h post-E2020 dosing, for assessment of E2020 and metabolite concentrations.

Safety was assessed by routine haematology and biochemistry at screening, pre-E2020 dose and post-study; by 12 lead ECG monitoring pre and post-study; and by blood pressure and heart rate measurement pre-study, prior to dosing, serially for 384 h post- dosing and post-study.

The pharmacokinetic parameters C_{max}, t_{max}, and AUC_{0-t} were calculated for each volunteer. A comparison of the parameters C_{max} and AUC_{0-t} was made between the two volunteer groups, using a two-sided students t-test. These parameters were also regressed against the calculated creatinine clearances for each volunteer, to assess whether there was any effect of renal function on the pharmacokinetics of E2020

Trial Population: Eight volunteers were recruited, of which eight successfully completed all phases of the study.

Volunteers were 4 patients with stable renal function impairment attending either Manchester Royal Infirmary or Withington Hospital renal units and 4 healthy age and sex matched controls

Results: There were 8 adverse events possibly related to study drug. Of these, 4 were headaches, 3 were diarrhoea/loose stool and 1 was nausea. Only 1 of these adverse events occurred in a renal patient.

TABLE 7

Preliminary pharmacokinetic parameters in 4 volunteers with moderate to severe renal impairment

SUBJECT	Cr CL *	C_{max} ($ng \cdot mL^{-1}$)	t_{max} (h)	AUC 0-t ($ng \cdot h \cdot mL^{-1}$)
1				
2				
3				
4				
MEAN	16.0	7.3	3.4	490
MEDIAN			4	
SD	4.9	2.8	0.8	66
CV%	31	39	24	14
MIN	9.7	4.8	2.5	416
MAX	21.4	10.5	4.0	577

TABLE 8

Preliminary pharmacokinetic parameters in 4 healthy volunteers

SUBJECT	Cr CL *	C_{max} ($ng \cdot mL^{-1}$)	t_{max} (h)	AUC 0-t ($ng \cdot h \cdot mL^{-1}$)
13				
14				
15				
16				
MEAN	89.0	7.6	3.2	435
MEDIAN			3	
SD	11.9	0.7	1.6	44
CV%	13	9	49	10
MIN	74.5	6.8	2.5	394
MAX	100.5	8.3	4.0	498

* creatinine clearance ($mL \cdot min^{-1}$ / 1.73 m^2) - average of screening and day 1 values

‡ (AUC_{0-t} including 312 h data = 733 $ng \cdot h \cdot mL^{-1}$)

FIGURE 2

Plot of mean (± sd) E2020 plasma concentration *versus* time (h) for renally impaired volunteers (Subs 1-4) and healthy volunteers (Subs 13-16)

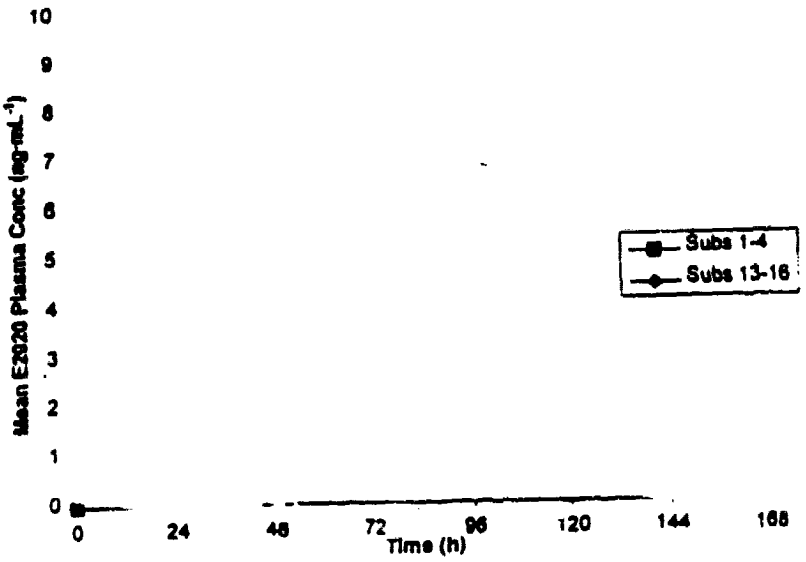


FIGURE 3

Effect of Creatinine Clearance (CrCL) on calculated pharmacokinetic (AUC_{0-t} and C_{max})

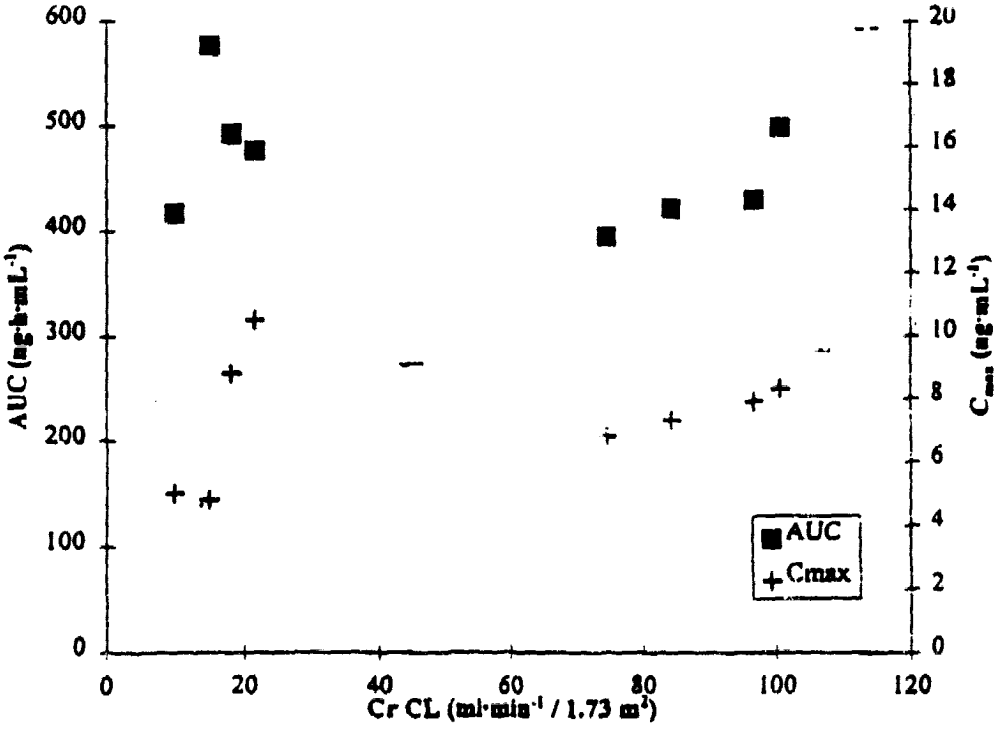


TABLE 5

Adverse Events

Subject no.	Initials	Study day	Adverse event	Start date & time	Duration	Severity	Relation to study medication	Action taken	Outcome
02									
13									
14									
15									
16									

Severity: 1=Mild 2=Moderate 3=Severe
Relation to study drug: 1=Not related 2=Possible 3=Definite
Action taken: 1=None 2=Test drug discontinued 3=Test drug dose reduced
Outcome: 1=Recovered-no residual effects 2=AE still present-no treatment 3=AE still present-being treated 4=Residual effects-no treatment 5=Residual effects-being treated 6=Lost to follow-up 7=Other

EFFECT OF GENDER

Tabel 1: Mean (±SD) Model Independent Pharmacokinetic parameters for E2020

Subjects	Cmax (ng/mL)	Tmax (hrs)	$AUC_{0-\infty}$ (ng*hr/mL)	Half-Life (hrs)	Clearance L/hr/kg
Males (N=7)	6.8 ± 1.5	5.0 ± 1.0	323 ± 65	68 ± 23	0.15 ± 0.04
Females (N=8)	8.0 ± 1.0	4.4 ± 1.8	377 ± 87	86 ± 19	0.16 ± 0.04

Elderly Study

CLINICAL STUDY SUMMARY

Title: A single-dose study to evaluate the safety, tolerance and pharmacokinetic profile of E2020 in elderly subjects.

Principal Investigator

Objectives:

- To evaluate the safety and tolerance of E2020 after a single, oral administration of 2 mg of E2020 to elderly subjects aged 65 to 84 years, and

- To establish the pharmacokinetic profile of E2020 after a single, oral administration of 2 mg of E2020 to elderly subjects aged 65 to 84 years.

Study Design: This investigation was designed to examine the effects of a single, oral administration of E2020 to elderly, Japanese subjects aged 65 to 84 years. The six subjects treated* received a single dose of 2 mg of E2020 orally after breakfast.

Methods: A single-dose study was performed in order to evaluate the safety and pharmacokinetics of E2020 in elderly subjects. After a standard breakfast, subjects were administered orally a single 2-mg dose of E2020. The safety evaluation of E2020 was based upon the assessment of the results of the following: physical examinations, recording of subjective and objective symptoms, vital signs, electrocardiogram recordings, and clinical laboratory tests.

Concentrations of E2020 in plasma, and unchanged parent drug and derived metabolites in urine, were determined by a sensitive and specific procedure. Blood samples were collected at selected time intervals for 7 days following the single administration of E2020. Urine samples were collected daily for 7 days post-dose. In addition, the binding of E2020 to proteins in plasma obtained from each subject was determined *ex vivo*.

* The protocol stated that eight subjects were to be enrolled; however, enrollment was terminated after the enrollment of seven subjects because of difficulty finding suitable subjects. One subject (Subject 3) was withdrawn before receiving active drug. The remaining 6 subjects completed the study.

Study Population: A total of 6 elderly, Japanese subjects (3 men and 3 women) aged 65 to 82 were treated in this study. The subjects were inpatients in [redacted] undergoing evaluation and treatment for various medical illnesses.

Results:

Safety:

E2020 was generally well tolerated by all subjects. Only one complaint was reported which was temporally associated with E2020 dosing. Subject 4 complained of mild malaise starting on Day 2 approximately 21 hours after dosing and lasting for 72 hours. No physical findings were noted with this symptom. The investigator concluded that a relationship of this symptom to E2020 could not be excluded.

No clinically significant changes were observed in vital signs or electrocardiograms. Most of these abnormalities occurred prior to dosing and all were considered unrelated to E2020.

Numerous minor clinical laboratory assessment abnormalities were noted during the study. All were considered clinically insignificant.

Pharmacokinetics:

The following plasma pharmacokinetic parameters for E2020, measured as free base, are listed in the table that follows: maximum concentration (C_{max}), time of C_{max} (T_{max}), plasma half-life ($t_{1/2}$), area under the plasma concentration time curve from zero to infinity ($AUC_{0-\infty}$) and clearance unadjusted for oral bioavailability (Cl/F).

Table 1 Background Data of Subjects

Subject (No.)	Sex	Age (year)	Height (cm)	Weight (kg)	Medical history	Present illness	Intelligence Evaluation
1							
2							
4							
5							
6							
7							
Mean		74.0	149.8	55.7			
±SD		7.80	9.72	4.46			

Noncompartmental Pharmacokinetic Parameters for E2020 (Measured as Free Base)

Subject No.	C_{max} (ng/ml)	T_{max} (hr)	$t_{1/2}$ (hr)	$AUC_{0-\infty}$ (ng x hr/nl)	Cl/F (l/hr)
1					
2					
4					
5					
6					
7					
Mean	3.07	5	136	271	7.16
SD	±1.00	±3	±34	±61	±2.42

On average, 94% of plasma E2020 was protein bound at 3 hours after dosing.

Urinary excretion of E2020, its metabolites and glucuronides of M-1 and M-2 over the 7 days following dosing is summarized in the following table.

Cumulative Urinary Excretion of E2020 and its Metabolites over 7 Days Expressed as a Percent of Dose

Subject	E2020	M-1	M-2	M-4	M-1 Glucuronide	M-2 Glucuronide
1						
2						
4						
5						
6						
7						
Mean	10.69	0.01	0.01	6.76	~3.74	1.72
SD	±10.84	±0.03	±0.02	±6.02	±1.77	±1.79

A total of about 23.% of the dose was recovered in urine during the 7 days after dosing. However, urine E2020 was still detectable in the last urine collection (144 - 168 hours) suggesting that significant urinary excretion continues after 7 days.

Table 9 Plasma Concentrations of E2020* and Pharmacokinetic Parameters

Dose 2.0mg
Plasma Concentration (ng/ml)

No.	0	1hr	2hr	3hr	4hr	6hr	10hr	24hr	48hr
±S.D.	0.00	0.41	1.14	1.43	0.64	0.42	0.25	0.22	0.15
±S.E.	0.00	0.17	0.46	0.58	0.26	0.17	0.10	0.09	0.06

No.	72hr	120hr	168hr	Cmax	Tmax	t1/2	AUC(Obs.)	AUC(Inf.)	CL/F
±S.D.	0.11	0.30	0.31	1.00	2.79	34.40	37.98	61.29	2.41
±S.E.	0.05	0.12	0.12	0.41	1.14	14.04	15.51	25.02	0.99
t1/2 terminal point (3)				(ng/ml)	(hr)	(hr)	(ngxhr/ml)	(ngxhr/ml)	(l/hr)

Values used for calculations carried greater decimal place accuracy than those reported in the tables; apparent discrepancies are due to round-off error.

*: For E2020 Free Base

Tabel 1: Mean (±SD) Model Independent Pharmacokinetic parameters for E2020

Subjects	Cmax (ng/mL)	Tmax (hrs)	$AUC_{0-\infty}$ (ng*hr/mL)	Half-Life (hrs)
Elderly	3.1 ± 1.0	5.0 ± 3.0	271 ± 61	136 ± 34
Young	3.2 ± 0.3	3.4 ± 1.5	247 ± 57	92 ± 33

Japanese Study

CLINICAL STUDY SUMMARY

Title: A single ascending oral dose study to evaluate the safety, tolerance and pharmacokinetic profile of E2020 in healthy male subjects.

Principal Investigator:

Objectives:

- To evaluate the safety of E2020 after single, oral administration in healthy male subjects, and
- To establish the pharmacokinetic profile of E2020 after single, oral administration in healthy male subjects.

Study Design: This randomized, single-blind, placebo-controlled, escalating dose, crossover study was designed to evaluate the effects of single oral doses of E2020 in healthy male subjects. The dose levels were 0.3 mg, 1 mg, 2 mg, 5 mg, 8 mg and 10 mg.

Methods: Eighteen subjects were randomly divided into 2 groups of 9 subjects each and the 2 groups participated alternately in successive steps of the study beginning with the lowest dose. In each step, 6 subjects took active compound and 3 took placebo (except in the 5-mg-dose study where 2 subjects took placebo). For each group, there was a washout period of at least 2 weeks between doses. Two additional subjects were enrolled to the study to replace the subjects who dropped out for reasons unrelated to E2020. Therefore total of 20 subjects enrolled in the study.

The safety evaluation of E2020 was based upon the assessment of the results of the following: physical examinations, recording of subjective symptoms, psychiatric evaluations, electrocardiograms, vital signs, echocardiograms, carotid pulse tracings, ophthalmological

examinations, electroencephalograms, and clinical laboratory determinations. The concentrations of E2020 and its metabolites in the plasma, urine and feces were measured by a sensitive and specific method.

Study Population: A total of 20 healthy male subjects were enrolled into this study. All were Japanese and between the ages of 24 and 38. Two subjects dropped out before completing their participation for reasons unrelated to E2020 treatment.

Results:

Safety:

E2020 was generally well tolerated by all subjects at each dose level. All adverse events were transient and of mild or moderate intensity. Multiple complaints (described below) which were considered related or possibly related to E2020 occurred in Step V (10-mg dose), resulting in a decrease in the planned Step VI dose from 20 mg to 8 mg. These complaints were: mild nausea in 3 subjects, mild weakness in 3 subjects, mild to moderate dizziness in 2 subjects, mild headache in 1 subject, increased bowel sounds in 1 subject, and a mild tremor in 1 subject. Additional complaints at the 10-mg dose that were considered possibly related to E2020 included soft stools in 2 subjects, stomach ache in 1 subject, diarrhea in 1 subject, sleepiness in 1 subject, a heavy-headed feeling in 1 subject, and myopia in 1 subject. At doses of up to 8 mg, fewer complaints were noted. Those considered related or possibly related to E2020 were: headache in 2 subjects (0.3-mg and 8-mg doses), abdominal pain in 2 subjects (1-mg and 2-mg doses), diarrhea in 2 subjects (1-mg and 2-mg doses), sleepiness in 1 subject (1-mg dose), a heavy feeling in the stomach in 1 subject (1-mg dose), and borborygmus in 1 subject (1-mg dose). No clinically significant changes were observed with any of the E2020 doses in vital signs, clinical laboratory tests, electrocardiograms, echocardiograms, carotid pulse tracings, electroencephalograms or ophthalmological examinations.

Pharmacokinetics:

Relevant model-independent plasma pharmacokinetic parameters are shown in the table that follows.

After administration of 2, 5, 8 and 10 mg E2020 doses, AUC increased linearly with dose within these dose ranges. But C_{max} increased by proportionately more than the increase in dose, and t_{max} became shorter as the dose increased. One possible reason for this is the increase in gastrointestinal motility secondary to the pharmacological action of E2020. The clearance and $t_{1/2}$ did not change with dose.

Pharmacokinetic parameters of E2020

Compound	Dose (mg)	C_{max} (ng/ml)	t_{max} (hr)	AUC [1] (ng × hr/ml)	$t_{1/2}$ [2] (hr)	CL/F (l/hr)
E2020	2	2.75 ± 0.49	3.67 ± 1.97	165.80 ± 30.27	51.4 ± 8.8	11.29 ± 1.91
	5	9.10 ± 1.90	3.00 ± 1.10	539.89 ± 142.21	52.8 ± 14.1	8.97 ± 2.44
	8	17.84 ± 4.30	2.58 ± 1.11	773.73 ± 240.93	51.7 ± 11.3	10.19 ± 2.98
	10	25.63 ± 8.95	2.42 ± 1.24	1002.19 ± 277.95	47.9 ± 9.8	9.67 ± 2.50

Mean ± S.D.

1) Area under the concentration-time curve between time zero and time infinity. AUC between the time of the last non-zero concentration (Cz) and time infinity was calculated by Cz/(terminal elimination rate constant). This terminal elimination rate constant was evaluated from the terminal three non-zero concentrations.
2) Determined from a two-compartment model.

E2020 metabolites could not be detected in the plasma.

Administered E2020 was excreted into the urine as unchanged E2020 and its metabolites, M-1, and its glucuronide, M-2 and its glucuronide, M-4 and M-6, and the mean (± S.D) urinary excretions through 264 hours after administration of a 10-mg dose are shown in the following table.

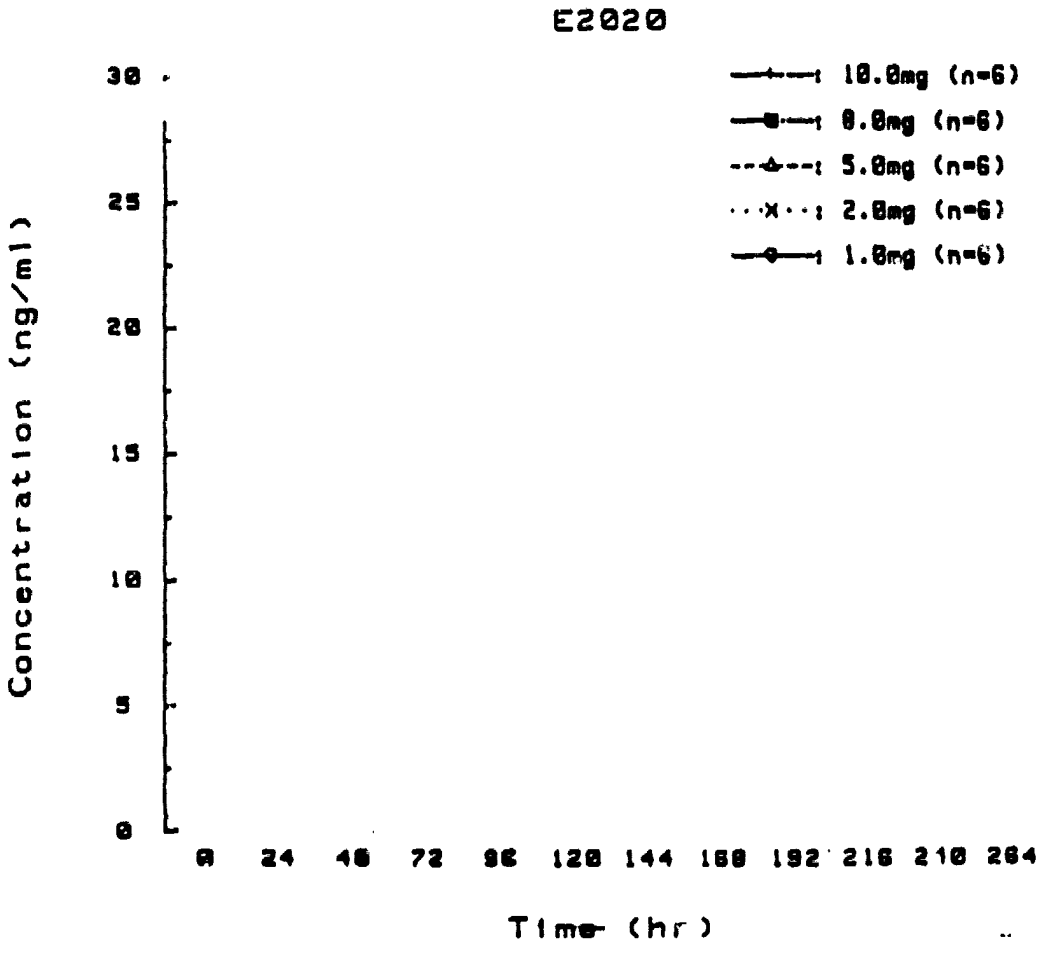


Fig.1-1 Mean Plasma Levels of E2020.
Each point represents the mean ± SE of 6 subjects.

EFFECTOR RACE

Tabel 1: Mean (±SD) Model Independent Pharmacokinetic parameters for E2020

Subjects	Cmax (ng/mL)	Tmax (hrs)	$AUC_{0-\infty}$ (ng*hr/mL)	Half-Life (hrs)	Clearance L/hr/kg
Japanese	9.1 ± 1.9	3.0 ± 1.0	540 ± 142	53 ± 14	0.14 ± 0.04
Cauc[illegible]ans	7.3 ± 1.5	4.7 ± 1.5	498 ± 136	78 ± 22	0.16 ± 0.04

123

Cimetidine

CLINICAL STUDY SYNOPSIS

Title: A Multiple-Dose Study of the Pharmacokinetics of E2020, An Acetylcholinesterase Inhibitor, and Cimetidine, in Healthy Volunteers; Study #: E2020-A001-006

Investigator:

Objective: The objective of this study was to examine the pharmacokinetics of E2020 and cimetidine separately, and in combination, following administration of single and multiple oral doses. Pharmacokinetic interactions (ie; changes in plasma concentrations of either drug), following concommitant administration of these drugs were also assessed.

Study Design: This study was a randomized, multiple-dose, open label, three-period crossover study which was conducted at a single investigational site.

Study Population: Nineteen (19) healthy, male subjects were enrolled into this study. A total of 18 subjects successfully completed the study. All participants were caucasian and ranged in age from 19 to 42 years (mean ± SD: 28 ± 7.6 years) and in weight from 66.5 to 85.0 kg (mean ± SD: 77.6 ± 5.0 kg).

Treatment Groups: The three treatments administered in this study were: 1) E2020, 5-mg tablet; 2) cimetidine, 800-mg tablet; 3) E2020, 5-mg + cimetidine 800-mg. Medication was administered as single, daily doses for 7 consecutive days, for each of the three periods. Medication was administered after a fast of at least eight hours and was accompanied by 250 ml of tap water.

Methods *(Clinical)*: Volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to the start of the study. For each Treatment period, subjects were admitted to the study site on the evening of day 0, at least 12 hours prior to drug administration. Upon admission, a routine physical examination was conducted. Entry vital signs (sitting position), and weight were recorded, and urine specimens for drug use/abuse screening were collected. The following morning, prior to drug administration, clinical laboratory and analytical samples were collected. Following drug administration, blood samples for analytical determinations were collected at specified intervals for 24 hours. Subjects were then discharged from the study site after providing a 24-hour blood sample. Prior to discharge, subjects received their second dose of medication for that period. Subjects returned to the site as outpatients for the next four mornings for additional analytical sample collection and to receive their daily

doses of medication. Subjects were readmitted to the study site on the evening of Day 6 and repeated the same schedule of events noted above for Day 0. Subjects were then discharged from the site on the morning of Day 8, 24-hours after receiving their final dose of medication for that Treatment period. Volunteers then returned to the study site at 24-hour intervals (to 168 hours), for additional analytical sample collection. Total in-house time for each treatment period was approximately 72-hours. Each Treatment period was followed by a 3-week drug-free, washout period.

Methods *(Analytical)*: Concentrations of E2020 and cimetidine in plasma were determined by [redacted]. The limits of quantitation for the E2020 assay [redacted]. The validated limits of quantitation for the cimetidine assay was [redacted]. E2020 and cimetidine pharmacokinetics were characterized using the resultant analytical data and standard techniques. Comparisons were made between groups for the Day 1 and Day 7 pharmacokinetic profiles. Parameters used for between-treatment comparisons included AUC_{0-24}, C_{max}, t_{max}, $t_{1/2}$ and the drug accumulation ratio, R_A.

Results: Pharmacokinetics - E2020: On Day 1, a statistically significant difference was observed between the E2020 group and the E2020 + cimetidine group in AUC_{0-24} ($p=0.032$) and C_{max} ($p=0.001$). The combination group showed a 10% greater AUC_{0-24} (102 ng*hr/ml vs 112 ng*hr/ml) and a 13% greater C_{max} (6.8 ng/ml vs 7.8 ng/ml) compared to the E2020 alone group. No significant difference was observed between the two groups in t_{max}. No significant sequence or period effects were observed. These results are presented in the table below:

Comparison of E2020 PK Parameters on Day 1 (Mean ± SE)

	C_{max} (ng/ml)	t_{max} (hrs)	AUC (0-24) (ng*hr/ml)
E2020	6.8 (0.4)	3.9 (0.3)	102.0 (5.8)
E2020 +Cimetidine	7.8 (0.4)	3.6 (0.3)	112.3 (4.5)
P-value	0.001	0.256	0.032

On Day 7, a statistically significant difference was again observed between the E2020 group and the E2020 + Cimetidine group in AUC_{0-24} ($p=0.0001$) and C_{max} ($p=0.0002$). The E2020 + Cimetidine group showed a 10% greater AUC_{0-24} (472.3 ng*hr/ml vs 526.9 ng*hr/ml) and a 11% greater C_{max} (26.6 ng/ml vs 29.9 ng/ml)

compared to the E2020 alone group. No significant difference was observed between the two groups in t_{max}, $t_{1/2}$, or in the rate of drug accumulation (R_A). No significant sequence effects were observed. These results are shown in the table below:

Comparison of E2020 PK Parameters on Day 7 (Mean ± SE)

	C_{max} (ng/ml)	t_{max} (hrs)	AUC (0-24) (ng*hr/ml)	$t_{1/2}$	R_A
E2020	26.6 (1.5)	4.5 (0.2)	472.3 (20.9)	64.9 (2.3)	4.8 (0.3)
E2020 + Cimetidine	29.9 (1.3)	4.4 (0.1)	526.9 (24.3)	62.0 (2.7)	4.7 (0.2)
P-value	0.0002	0.664	0.0001	0.151	0.806

Although specific PK parameters were found to be statistically different between the E2020 and the E2020 + Cimetidine groups, all differences were within the ± 20% range for both Days 1 and 7.

Pharmacokinetics - Cimetidine: On Day 1, a statistically significant difference was observed between the cimetidine group and the E2020 + cimetidine group in C_{max} (p=0.0011). The combination group showed a 20% greater C_{max} (4.1 ± 0.3 ng/ml vs 3.3 ± 0.2 ng/ml) than the cimetidine alone group. No statistically significant difference was observed in AUC_{0-24} or t_{max} between the two groups.

On Day 7, no statistically significant difference was observed between the two groups in C_{max}, AUC_{0-24}, $t_{1/2}$, or in the rate of drug accumulation (R_A). The cimetidine alone group did, however, show a statistically significant greater t_{max} (p=0.016) than did the combination group (3.7 ± 0.2 hrs vs 3.1 ± 0.2 hrs). No significant sequence effects were observed.

Although specific PK parameters were found to be statistically different between the cimetidine and the E2020 + Cimetidine groups, in general, no specific trends or changes in cimetidine pharmacokinetics were observed between the 2 groups.

Safety: No serious or unexpected adverse experiences occurred during the course of this study. Treatment Emergent Signs and Symptoms (TESS) were transient and the majority were mild or moderate in severity. The incidence and severity of TESS for E2020 and cimetidine administered in combination, were similar to those for E2020 administered alone.

No clinically significant, treatment emergent abnormal laboratory values (TEAV's) were observed during the course of this study.

110 (Replacement subject) Meal scheduled for 20:20 (-10.5 hours, Treatment period 1) on 3-11-95, was eaten 17 minutes, 49 seconds early.

110 Dosing report 06:50 (24 hours, Treatment period 2), 4-10-95; dose administration was late by 1 minute.

5.4 PHARMACOKINETICS

5.4.1 E2020

Mean plasma E2020 concentrations are presented by timepoint for each E2020 Treatment group (E2020 alone, E2020 + Cimetidine) in TABLE 7 (data by individual subject can be found in Data Listing 16). A time versus concentration plot of this mean data is presented in FIGURE II.

The complete results of the pharmacokinetic analyses are presented in TABLE 9 and several parameters are summarized in the table below. On Day 1, a statistically significant difference was observed between the E2020 group and the E2020 + Cimetidine group in AUC_{0-24} (p=0.0321) and C_{max} (p=0.0011). The E2020 + Cimetidine group showed a 10% greater AUC_{0-24} (102 ng*hr/ml vs 112 ng*hr/ml) and a 13% greater C_{max} (6.8 ng/ml vs 7.8 ng/ml) compared to the E2020 alone group. The coefficient of variation (CV) was 12.2% and 10.4% for AUC_{0-24} and C_{max}, respectively. No significant difference was observed between the two groups in t_{max}. No significant sequence effects were observed.

Comparison of E2020 PK Parameters on Day 1 (Mean ± SE)

	C_{max} (ng/ml)	t_{max} (hrs)	AUC (0-24) (ng*hr/ml)
E2020	6.8 (0.4)	3.9 (0.3)	102.0 (5.8)
E2020 + Cimetidine	7.8 (0.4)	3.6 (0.3)	112.3 (4.5)
P-value	0.001	0.256	0.032

On Day 7, a statistically significant difference was again observed between the E2020 group and the E2020 + Cimetidine group in AUC_{0-24} (p=0.0001) and C_{max} (p=0.0002). As shown in the table below, the E2020 + Cimetidine group showed a 10% greater AUC_{0-24} (472.3 ng*hr/ml vs 526.9 ng*hr/ml) and a 11% greater C_{max} (26.6 ng/ml vs 29.9 ng/ml) compared to the E2020 alone group. The coefficient of variation (CV) was 6.0% and 7.0% for AUC_{0-24} and C_{max}, respectively. No significant difference was observed between the two groups in t_{max}, $t_{1/2}$, or in the rate of E2020 accumulation (R_A). No significant sequence effects were observed.

Comparison of E2020 PK Parameters on Day 7 (Mean ± SE)

	C_{max} (ng/ml)	t_{max} (hrs)	AUC (0-24) (ng*hr/ml)	$t_{1/2}$ (hrs)	RA
E2020	26.6 (1.5)	4.5 (0.2)	472.3 (26.9)	61.9 (2.3)	4.8 (0.3)
E2020 + Cimetidine	29.9 (1.3)	4.4 (0.1)	526.9 (24.3)	62.0 (2.7)	4.7 (0.2)
P-value	0.0002	0.664	0.0001	0.151	0.806

All differences observed in the pharmacokinetic parameters for the E2020 group and the E2020 + Cimetidine group were within ± 20% for both the Day 1 and Day 7 comparisons. The means by sequence and by period are presented in APPENDIX VIII along with pharmacokinetic parameters by subject and details of the statistical analysis.

5.4.2 Cimetidine

Mean plasma cimetidine concentrations are presented by timepoint for each cimetidine treatment group (cimetidine alone, E2020 + cimetidine) in DATA LISTING 17. A time versus concentration plot of this mean data is presented in FIGURE III.

The complete results of the cimetidine pharmacokinetic analyses are presented in TABLE 10 and several parameters are summarized in the tables below. On Day 1, a statistically significant difference was observed between the cimetidine group and the E2020 + Cimetidine group in C_{max} ($p=0.0011$). The combination group showed a 20% greater C_{max} (4.1 ng/ml vs 3.3 ng/ml) than the cimetidine alone group. No statistically significant difference was observed in AUC_{0-24} or t_{max} between the two groups.

Comparison of Cimetidine PK Parameters on Day 1 (Mean ± SE)

	C_{max} (ng/ml)	t_{max} (hrs)	AUC (0-24) (ng*hr/ml)
Cimetidine	3.3 (0.2)	2.7 (0.4)	17.7 (0.8)
E2020 + Cimetidine	4.1 (0.3)	2.5 (0.3)	18.9 (0.9)
P-value	0.001	0.435	0.057

In contrast, no statistically significant difference in C_{max} was observed between the two groups on Day 7, nor was there any difference in AUC_{0-24}, $t_{1/2}$ or accumulation ratio (R_A). The cimetidine alone group did show a statistically significant greater t_{max}

(p=0.016) than did the combination group (3.7 hrs vs 3.1 hrs, respectively). No significant sequence effects were observed.

Comparison of Cimetidine PK Parameters on Day 7 (Mean ± SE)

	C_{max} (ng/ml)	t_{max} (hrs)	AUC (0-24) (ng*hr/ml)	$t_{1/2}$ (hrs)	RA
Cimetidine	4.0 (0.3)	3.7 (0.2)	20.6 (1.0)	3.0 (0.2)	1.2 (0.0)
E2020 + Cimetidine	4.4 (0.4)	3.1 (0.2)	20.4 (0.8)	3.3 (0.3)	1.1 (0.0)
P-value	0.162	0.016	0.762	0.227	0.211

All differences observed in the pharmacokinetic parameters for the cimetidine and the E2020 + cimetidine groups were within ± 20% for both the Day 1 and Day 7 comparisons. The means by sequence and by period are presented in APPENDIX IX along with pharmacokinetic parameters by subject and details of the statistical analysis.

5.5 SAFETY EVALUATIONS

5.5.1 Laboratory Evaluations: Standard laboratory evaluations were conducted at Screening, upon admission to the study site for each Treatment period, and at the time of Study Discharge. A treatment emergent abnormal value (TEAV) for a laboratory parameter is defined as a value that was below the lower limit of the normal range post-dose but was not below it prior to dose administration, or a value that was above the upper limit of the normal range post-dose, but was not above it prior to dose administration. The number of TEAV's noted during the course of this study were minimal, and are listed by Treatment group in TABLE 4. In addition, the Investigators comments on each TEAV is presented in DATA LISTING 9.

A summary of all the clinical laboratory values obtained for each subject during the study is presented in DATA LISTING 6.

5.5.2 Routine Physical Examinations General well being was assessed by a brief evaluation of the head, eyes, ears, nose, throat and other physical conditions of note upon admission to the study site for each Treatment period. No abnormal changes in general physical well being were noted for any subject during any Treatment period. A tabulation of Routine Physical Examination results is provided in DATA LISTING 4.

5.5.3 Electrocardiogram

All 12 subjects enrolled in the study had an ECG examination at the time of Screening and again at the time of Final discharge. No changes in ECG patterns were noted for

any subject. One subject had a left anterior fasicular block at Screening that was also observed at Final discharge (left axial deviation). There were mild variations in heart rate between Screening and Final discharge for all of the subjects, but there was no discernible trend to these fluctuations and they were judged by the Investigator not to be clinically relevant. The electrocardiogram data is presented in DATA LISTING 5.

5.5.4 Vital Signs

DATA LISTING 11 lists the vital signs for all subjects during each of the three treatment periods. The mean changes from baseline for radial pulse rate, systolic and diastolic blood pressure for each treatment group are presented in TABLE 3 and displayed in FIGURES IV, VI and VIII (Day 1), and FIGURES V, VII and IX (Day 7). These results indicate that the coadministration of E2020 and cimetidine does not influence blood pressure or pulse rate to a greater degree than the administration of E2020 or cimetidine alone.

5.5.5 Adverse Events

No serious or unexpected adverse events were reported during this study. Of the 19 subjects enrolled, 12 reported experiencing an adverse event at some time during the course of the study. The total number of events documented over the 10-week study period was 66. All reported adverse events were mild to moderate in severity. Of the 66 reported adverse events, 36 were considered "possibly related" to the drug being administered, 30 were considered "not related", and none were considered "definitely related". All adverse events that were experienced during this study are presented in DATA LISTING 13.

Those events which took place after dose administration were classified as Treatment Emergent Signs and Symptoms (TESS) and are summarized in TABLE 5.

Table 9
Pharmacokinetic Parameters for E2020

		E2020 5mg	E2020 5mg + Cimetidine 800mg	P-value
Day 1				
$AUC_{0\text{-}24\ hrs.}$ (ng*hr/mL)				
	N	18	18	
	Mean (S.E.)	102.0 (5.8)	112.3 (4.5)	0.0321
	Min - Max	49.4 - 144.8	89.4 - 149.3	
t_{max} (Hour)				
	N	18	18	
	Mean (S.E.)	3.9 (0.3)	3.6 (0.3)	0.2560
	Min - Max	2.0 - 6.0	2.0 - 6.0	
C_{max} (ng/mL)				
	N	18	18	
	Mean (S.E.)	6.8 (0.4)	7.8 (0.4)	0.0011
	Min - Max	4.7 - 10.6	5.9 - 11.9	
Day 7				
$AUC_{0\text{-}24\ hrs.}$ (ng*hr/mL)				
	N	18	18	
	Mean (S.E.)	472.3 (26.9)	526.9 (24.3)	0.0001
	Min - Max	239.8 - 763.1	341.1 - 719.0	
t_{max} (Hour)				
	N	18	18	
	Mean (S.E.)	4.5 (0.2)	4.4 (0.1)	0.6642
	Min - Max	3.0 - 7.0	4.0 - 5.0	
C_{max} (ng/mL)				
	N	18	18	
	Mean (S.E.)	26.6 (1.5)	29.9 (1.3)	0.0002
	Min - Max	15.7 - 45.0	20.7 - 38.8	
$t_{1/2}$ (Hour)				
	N	18	18	
	Mean (S.E.)	64.9 (2.3)	62.0 (2.7)	0.1511
	Min - Max	46.2 - 79.7	45.4 - 85.0	
R_A				
	N	18	18	
	Mean (S.E.)	4.8 (0.3)	4.7 (0.2)	0.8068
	Min - Max	2.8 - 9.4	3.5 - 6.4	

Data Source: Statistical Appendix VIII

Table 10
Pharmacokinetic Parameters for Cimetidine

		Cimetidine 8mg	E2020 5mg + Cimetidine 800mg	P-value
Day 1				
$AUC_{0\text{-}24\ hrs.}$ (ng*hr/mL)				
	N	18	18	
	Mean (S.E.)	17.7 (0.8)	18.9 (0.9)	0.0570
	Min - Max	12.3 - 25.4	13.7 - 28.6	
t_{max} (Hour)				
	N	18	18	
	Mean (S.E.)	2.7 (0.4)	2.5 (0.3)	0.4353
	Min - Max	0.5 - 6.0	1.0 - 6.0	
C_{max} (ng/mL)				
	N	18	18	
	Mean (S.E.)	3.3 (0.2)	4.1 (0.3)	0.0011
	Min - Max	1.9 - 5.8	2.5 - 6.9	
Day 7				
$AUC_{0\text{-}24\ hrs.}$ (ng*hr/mL)				
	N	18	18	
	Mean (S.E.)	20.6 (1.0)	20.4 (0.8)	0.7628
	Min - Max	14.9 - 30.6	14.6 - 28.3	
t_{max} (Hour)				
	N	18	18	
	Mean (S.E.)	3.7 (0.2)	3.1 (0.2)	0.0160
	Min - Max	2.0 - 5.0	2.0 - 5.0	
C_{max} (ng/mL)				
	N	18	18	
	Mean (S.E.)	6.0 (0.3)	6.4 (0.4)	0.1622
	Min - Max	2.8 - 6.7	2.7 - 8.3	
$t_{1/2}$ (Hour)				
	N	18	18	
	Mean (S.E.)	3.0 (0.2)	3.3 (0.3)	0.2270
	Min - Max	1.8 - 4.7	2.1 - 6.4	
R_A				
	N	18	18	
	Mean (S.E.)	1.2 (0.0)	1.1 (0.0)	0.2110
	Min - Max	1.0 - 1.7	0.9 - 1.4	

Data Source: Statistical Appendix IX

Figure II
E2020 – A001 – 006

E2020 Plasma Concentration (ng/mL)

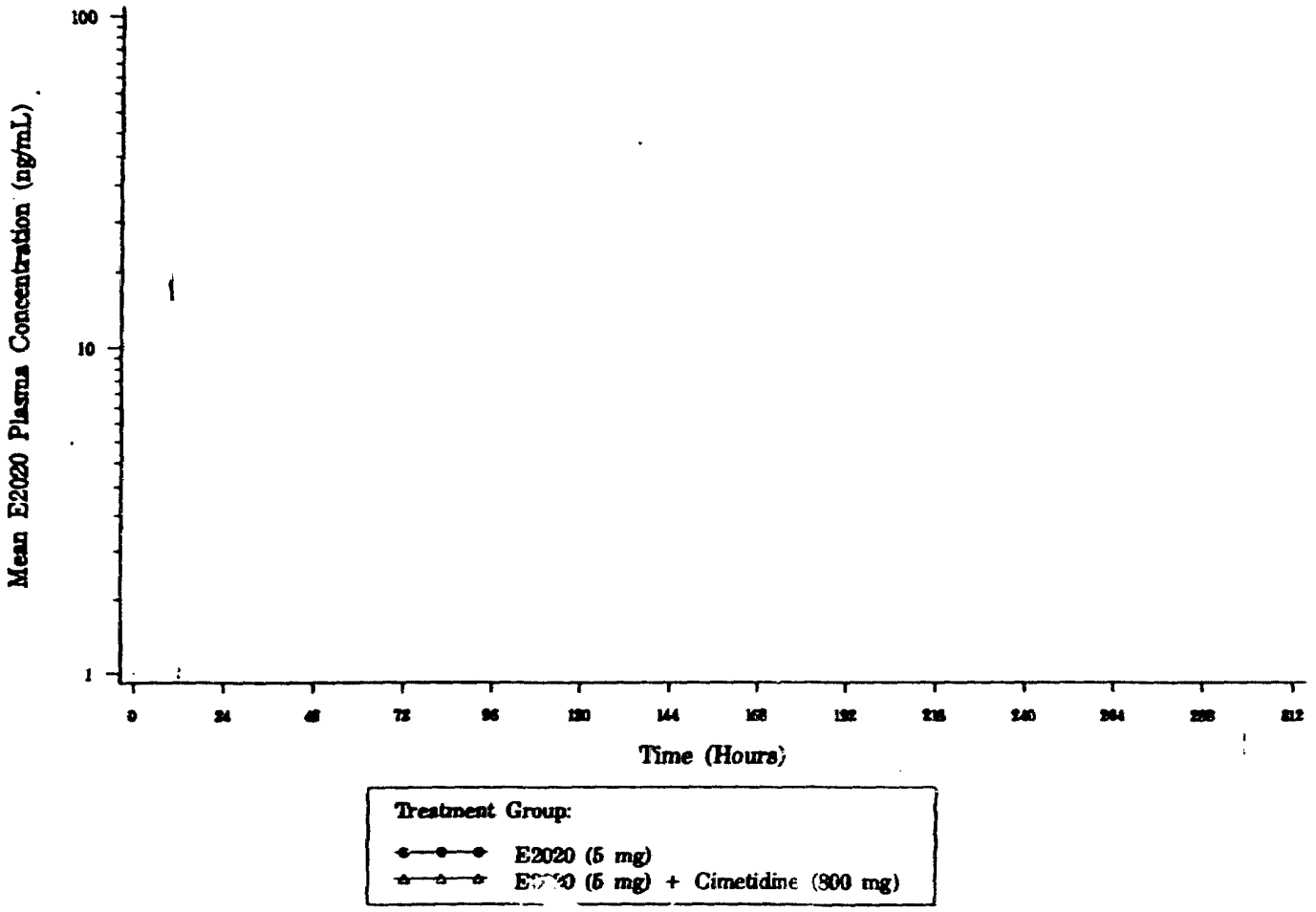
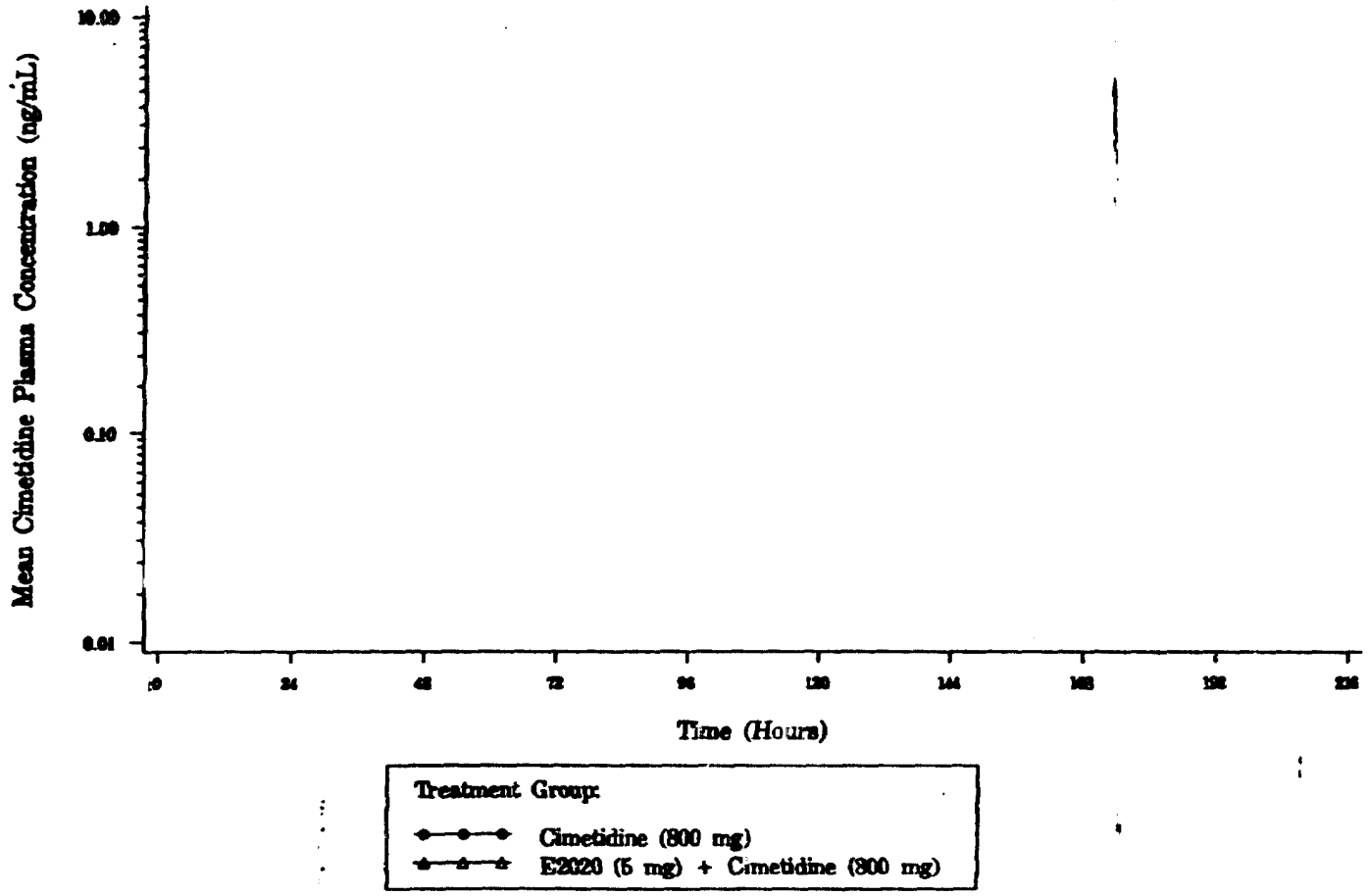


Figure III
E2020 – A001 – 006

Cimetidine Plasma Concentration (ng/mL)



Note: Concentrations were below detectable limit at 48, 192, and 216 hours.

CLINICAL STUDY SYNOPSIS

Title: A Single-Dose Study of the Pharmacokinetics of E2020, An Acetylcholinesterase Inhibitor, and Digoxin, in Healthy Volunteers; Study #: E2020-A001-007

Investigator:

Objective: The primary objective of this study was to examine the pharmacokinetics of E2020 and digoxin separately, and in combination, following administration of single oral doses. Pharmacokinetic interactions and changes in cardiac function following concommitant administration of these drugs were also assessed.

Study Design: This study was a randomized, single-dose, open label, three-period crossover study which was conducted at a single investigational site.

Study Population: Twelve healthy, male volunteers were randomized into this study and all twelve subjects completed the study without incident. Participants were Caucasian and ranged in age from 19 to 44 years (mean ± SD: 28 ± 7.6 years) and in weight from 66.5 to 85.0 kg (mean ± SD: 77.6 ± 5.0 kg).

Treatment Groups: The three treatments administered in this study were: 1) E2020, 5 mg tablet; 2) Digoxin, 0.25 mg tablet; 3) E2020, 5 mg + Digoxin, 0.25 mg. Medication was administered after a fast of at least eight hours and was accompanied by 250 ml of tap water.

Methods *(Clinical)*: Volunteers were screened by medical history, ECG, and laboratory and physical examinations ≤ 2 weeks prior to the start of the study. For each Treatment period, subjects were admitted to the telemetry unit of the study site on the evening of day 0, at least 12 hours prior to drug administration. Upon admission, a routine physical examination was conducted. Entry vital signs (sitting position), and weight were recorded, and urine specimens for drug use/abuse screening were collected. A light snack was served 10 hours prior to drug administration, after which an absolute fast, from both food and fluids (except water), was maintained. The following morning, ECG monitoring (Lead II) began one hour *prior* to drug administration, and continued for 24 hours after drug administration. Following drug administration, blood samples for analytical determinations were collected at specified intervals for 24 hours. Subjects were discharged from the study site the following morning after providing a 24-hour blood sample. Volunteers then returned to the study site at 24-hour intervals (to 120 hours), for additional analytical sample collection.

Methods *(Analytical)*: Concentrations of E2020 in plasma were determined by a) method (range of quantitatio Digoxin concentrations in plasma were determined by ng/ml). Characterization of E2020 and digoxin pharmacokinetics was conducted on the resultant analytical data using standard techniques. Parameters used for between-treatment comparisons included AUC $_{0\text{-}120}$, C_{max}, t_{max} and $t_{1/2}$.

Results:

Pharmacokinetics: No statistically significant differences in E2020 pharmacokinetics (AUC $_{0\text{-}120}$, C_{max}, t_{max} or $t_{1/2}$) were observed when E2020 administered alone was compared to E2020 administered concurrently with digoxin. Similarly, no significant differences in digoxin pharmacokinetics were observed when digoxin administered alone was compared with digoxin administered concurrently with E2020. These results are summarized in the tables below:

E2020 Pharmacokinetic Parameters (Mean ±S.E.)

	t_{max} (hrs)	C_{max} (ng/ml)	AUC $_{0\text{-}120}$ (ng*hr/ml)	$t_{1/2}$ (hrs)
E2020 (5-mg)	4.5 (0.4)	6.3 (0.3)	280.2 (20.6)	70.7 (7.2)
E2020 + Digoxin	4.5 (0.4)	6.3 (0.3)	275.0 (16.7)	74.0 (9.8)

Digoxin Pharmacokinetic Parameters (Mean ±S.E.)

	t_{max} (hrs)	C_{max} (ng/ml)	AUC $_{0\text{-}120}$ (ng*hr/ml)	$t_{1/2}$ (hrs)
Digoxin (0.25-mg)	1.3 (0.1)	0.8 (0.1)	9.7 (0.7)	56.4 (6.8)
Digoxin + E2020	1.2 (0.1)	0.8 (0.1)	8.7 (0.9)	59.9 (7.6)

Safety: No serious or unexpected adverse experiences occurred during the course of this study. Treatment Emergent Signs and Symptoms (TESS) were transient and the majority were mild or moderate in severity. The incidence and severity of TESS for E2020 and digoxin were similar for each drug when it was administered alone or in combination.

No clinically relevant changes in Lead II ECG (heart rate, duration of PR, QRS and QT intervals) were observed in any of the subjects, in

any of the treatment groups, during the 24-hours of monitoring following drug administration.

No clinically significant, treatment emergent abnormal laboratory values (TEAV's) were observed during the course of this study.

Conclusions:

The conclusions from this study are:

- That digoxin (0.25-mg) does not affect the pharmacokinetics of E2020 following single-dose administration.
- That E2020 (5-mg) does not affect the pharmacokinetics of digoxin following single-dose administration.
- That these two drugs were coadministered without any resulting changes in ECG parameters, or increases in the occurrence of TESS or treatment emergent abnormal laboratory values.

Table 9
E2020 Pharmacokinetic Parameters

		E2020 5mg	E2020 5mg + Digoxin .25mg	P-value	Estimate and 95% C.I.*
$AUC_{0\text{-}120\ hrs}$ (ng*hr/mL)					
	N	12	12		
	Mean (S.E.)	280.2 (20.6)	275.0 (16.7)	0.6113	5.197 (-9.28 , 19.67)
	Min - Max	158.6 - 422.7	194.3 - 386.8		
$AUC_{0-\infty}$ (ng*hr/mL)					
	N	12	12		
	Mean (S.E.)	425.3 (44.4)	417.3 (40.5)	0.6021	8.034 (-13.77 , 29.84)
	Min - Max	205.9 - 626.1	253.0 - 721.1		
t_{max} (Hour)					
	N	12	12		
	Mean (S.E.)	4.5 (0.6)	4.5 (0.4)	1.0000	0.000 (-0.77 , 0.77)
	Min - Max	3.0 - 6.0	3.0 - 8.0		
C_{max} (ng/mL)					
	N	12	12		
	Mean (S.E.)	6.3 (0.3)	6.3 (0.3)	0.9778	-0.007 (-0.36 , 0.36)
	Min - Max	4.9 - 8.4	6.5 - 8.9		
$t_{1/2}$ (Hour)					
	N	12	12		
	Mean (S.E.)	70.7 (7.2)	74.0 (9.8)	0.5566	-3.268 (-11.85 , 4.55)
	Min - Max	25.2 - 126.7	31.7 - 154.1		

Data Source: Statistical Appendix VIII

* *The difference in the lsmeans between the two treatment groups and the 95% confidence interval.*

Table 10
Digoxin Pharmacokinetic Parameters

		Digoxin .25mg	E2020 5mg + Digoxin .25mg	P-value	Estimate and (95% C.I.)*
$AUC_{0-120\,hrs}$ (ng*hr/mL)					
	N	12	12		
	Mean (S.E.)	9.7 (0.7)	8.7 (0.9)	0.2890	1.006 (-0.30 , 2.32)
	Min - Max	6.3 - 14.4	4.9 - 13.6		
$AUC_{0-\infty}$ (ng*hr/mL)					
	N	12	12		
	Mean (S.E.)	18.9 (1.8)	19.5 (2.1)	0.7971	-0.628 (-4.10 , 2.85)
	Min - Max	13.3 - 26.1	9.8 - 31.6		
t_{max} (Hour)					
	N	12	12		
	Mean (S.E.)	1.3 (0.1)	1.2 (0.1)	0.3558	0.167 (-0.08 , 0.42)
	Min - Max	1.0 - 2.0	1.0 - 2.0		
C_{max} (ng/mL)					
	N	12	12		
	Mean (S.E.)	0.8 (0.1)	0.8 (0.1)	0.7173	-0.021 (-0.19 , 0.06)
	Min - Max	0.3 - 1.2	0.5 - 1.3		
$t_{1/2}$ (Hour)					
	N	12	12		
	Mean (S.E.)	56.4 (6.8)	59.9 (7.6)	0.7546	-3.460 (-19.20 , 12.28)
	Min - Max	31.5 - 120.1	18.2 - 108.7		

Data Source: Statistical Appendix IX

* The difference in the lsmeans between the two treatment groups and the 95% confidence interval.

Figure II
E2020 – A001 – 007

E2020 Plasma Concentration (ng/mL)

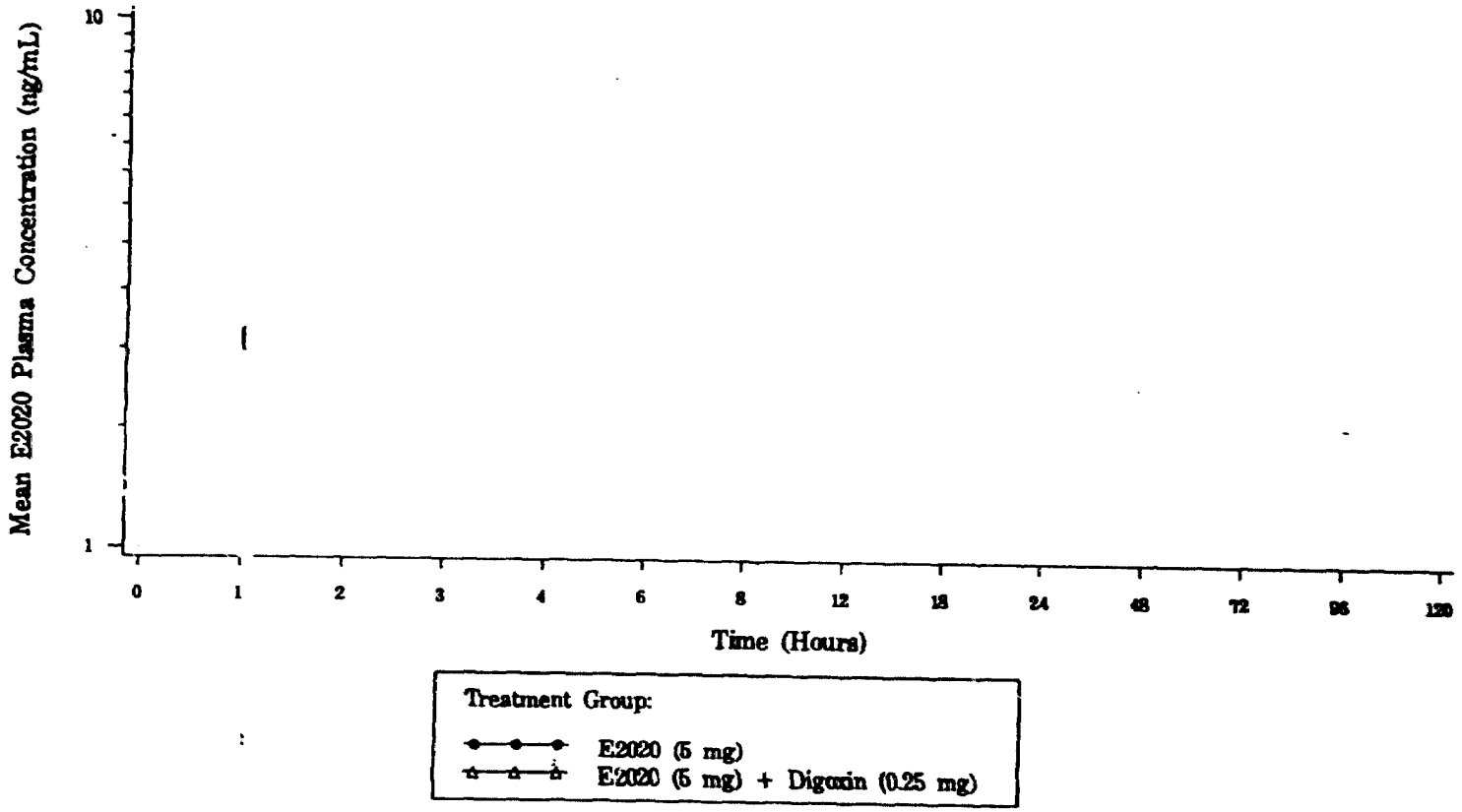
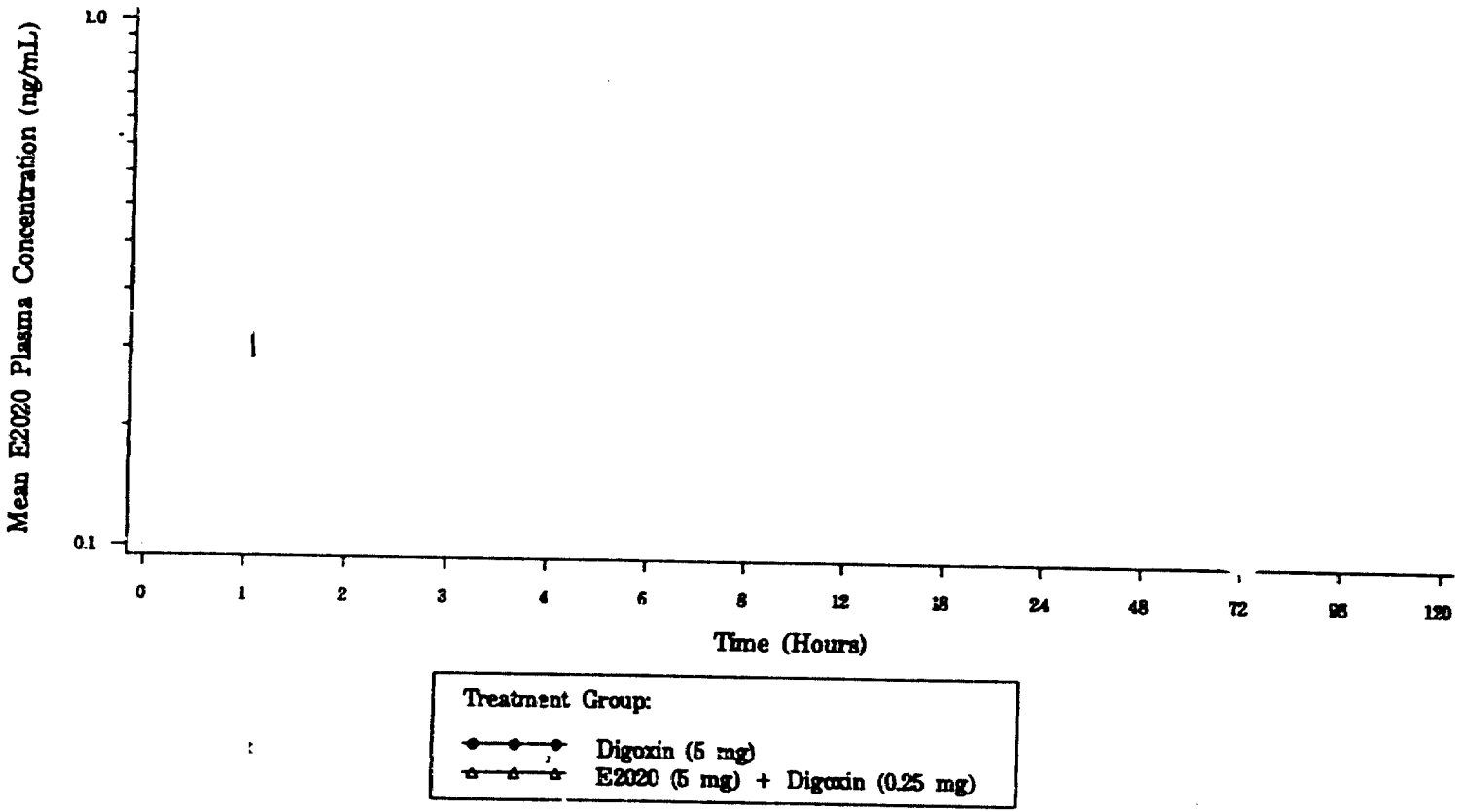


Figure III
E2020-A001-007

Digoxin Plasma Concentration (ng/mL)



SUMMARY / SYNOPSIS

8 Theophylline

Objectives: To evaluate any clinically relevant effects of multiple doses of E2020 on the pharmacokinetics of theophylline from a sustained release preparation. To examine the safety and tolerability of multiple doses of E2020 and sustained release theophylline given concurrently.

Trial Design and Methods: This was a two period, open-label randomised crossover study with an introductory period to determine the dose of theophylline needed to give therapeutic concentrations and assess tolerability to theophylline prior to the main part of the study. After the appropriate twice daily dose of theophylline had been selected, each volunteer received either A) theophylline alone or B) theophylline plus 5mg E2020 daily. Each session lasted ten days. There was a one week washout between the introductory period and the first session and a three week washout between the two treatment sessions.

Serial blood samples were collected over a 24 h period on Day 10, covering the final two theophylline doses, for assessment of plasma theophylline levels.

Safety was assessed by routine haematology and biochemistry at screening, prior to dosing on Days 10 and 11 of each study session and at the post-study review, by 12-lead ECG at screening and post-study review, by blood pressure and heart rate measurement at every visit and by remaining trough theophylline levels on Days 2, 4, 6, 7, 8 and 9 in each treatment session.

The pharmacokinetic parameters C_{max} and AUC_τ for each dosing interval on Day 10 (in 0-12 h and 12-24 h) were compared in the presence and absence of E2020 by analysis of variance. Wilcoxon's matched-pairs signed ranks test was used to compare t_{max} for each interval in the presence and absence of E2020.

Trial Population: Volunteers were non-smoking healthy males aged 19-36 years within 15% of normal weight for height an build, with no past medical history of clinical significance. Thirteen volunteers were recruited, of which twelve successfully completed all phases of the study.

Results: There were no statistically significant differences in the pharmacokinetics of theophylline in the presence or absence of E2020. Mean (± sd) results were:

Parameter		Interval	Theophylline Alone	Theophylline + E2020
C_{max}	($mg \cdot L^{-1}$)	0-12 h	11.45 (1.52)	11.01 (1.45)
t_{max}	(h) *	0-12 h	4 (2-8)	3 (0-8)
AUC_{0-12}	($mg \cdot h \cdot L^{-1}$)	0-12 h	112 (15)	113 (18)
C_{max} –	($mg \cdot L^{-1}$)	12-24 h	9.47 (1.59)	10.07 (1.09)
t_{max}	(h) *	12-24 h	8 (0-12)	8 (6-12)
AUC_{12-24}	($mg \cdot h \cdot L^{-1}$)	12-24 h	96 (19)	101 (12)

* median (range)

There were a small number of adverse events (22) possibly or definitely related to study medication of which 4 occurred during treatment with theophylline alone and 18 occurred during treatment with theophylline and E2020. There were no clinically significant changes in any of the safety parameters assessed.

Conclusions: E2020 did not affect the pharmacokinetics of theophylline.

Concurrent treatment with multiple 5mg doses of E2020 and sustained release theophylline was generally well tolerated and there were no clinically significant changes in the safety parameters assessed.

TABLE 7

Plasma theophylline pharmacokinetic parameters following a ten day multiple dose regime of theophylline alone in 12 healthy volunteers

SUBJECT	Period	0- 12 h			12 - 24 h		
		C_{max} ($mg \cdot L^{-1}$)	t_{max} (h)	AUC_{0-12} ($mg \cdot h \cdot L^{-1}$)	C_{max} ($mg \cdot L^{-1}$)	t_{max} (h)	AUC_{12-24} ($mg \cdot h \cdot L^{-1}$)
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
21							
MEAN		1.45		112	9.47		96
MEDIAN			4			8	
SD		1.52		15	1.59		19
CV%		13		14	17		20
MIN		9.45	2	81	6.21	0	70
MAX		14.09	8	145	12.05	12	129

TABLE 8

Plasma theophylline pharmacokinetic parameters following a ten day multiple dose regime of theophylline together with E2020 in 12 healthy volunteers

SUBJECT	Period	0- 12 h			12 - 24 h		
		C_{max} ($mg \cdot L^{-1}$)	t_{max} (h)	AUC_{0-12} ($mg \cdot h \cdot L^{-1}$)	C_{max} ($mg \cdot L^{-1}$)	t_{max} (h)	AUC_{12-24} ($mg \cdot h \cdot L^{-1}$)
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
21							
MEAN		11.01		113	10.07		101
MEDIAN			3			8	
SD		1.45		18	1.09		12
CV%		13		16	11		12
MIN		8.18	0	74	8.03	6	83
MAX		13.27	8	150	11.70	12	126

TABLE 9

Steady State Plasma E2020 Concentrations Obtained on Day 10 (0 h) and Day 11 (24 h) of a Multiple Dose Regime of E2020 5 mg Daily

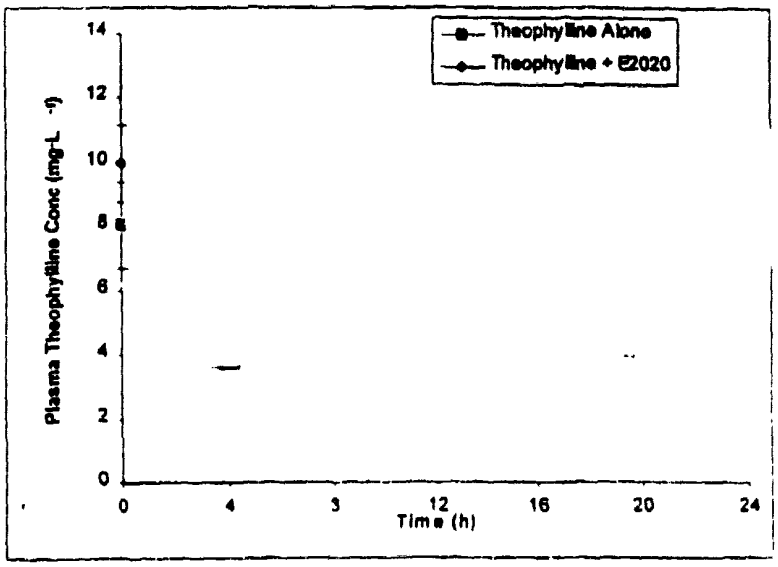
Subject	Concentration	Hour
009		0
009		24
010		0
010		24
011		0
011		24
012		0
012		24
013		0
013		24
014		0
014		24
015		0
0.15		24
016		0
016		24
017		0
017		24
018		0
018		24
019		0
019		24
021		0
021		24

at '0' hr
Mean C_{min} = 16.9 ± 5.7

at '24' hr
Mean C_{min} = 16.8 ± 6.0

FIGURE 2

Mean plasma theophylline concentration-time data following a ten day multiple dose regime of theophylline alone or theophylline with E2020 in 12 healthy volunteers



9

WARFARIN

SUMMARY / SYNOPSIS

Objectives: To evaluate any clinically relevant effects of multiple doses of E2020 on the pharmacokinetics and pharmacodynamics (as measured by prothrombin time) of a single dose of warfarin. To assess the safety and tolerability of multiple doses of E2020 and a single dose of warfarin given concurrently.

Trial Design and Methods: This was a two period, open label, randomised, balanced crossover study in 12 healthy males aged 18-55 years. Each volunteer received one of the following treatments:
Treatment Schedule A: E2020 for 19 days with a single dose of warfarin after 14 days. After a 21 day washout period warfarin was administered alone.
Treatment Schedule B: A single dose of warfarin administered alone initially. After a 7 day washout period, E2020 was administered for 19 days with a single dose of warfarin after 14 days treatment.

Serial blood samples were collected over 144 h post-warfarin dosing, for assessment of prothrombin time and plasma (R)-warfarin and plasma (S)-warfarin concentrations.

Safety was assessed by routine haematology and biochemistry at screening, pre-warfarin doses and post-study; by 12 lead ECG monitoring pre and post-study; and by blood pressure and heart rate measurement pre-study, prior to dosing, serially for 144 h post-warfarin dosing and post-study.

The pharmacokinetics parameters C_{max}, $AUC_{0-\infty}$, CL/F, V_z/F and $t_{½}$ for (R) and (S)-warfarin and the area under the prothrombin time curve (AUC_{PT}) and maximum prothrombin time (R_{max}) were compared in the presence and absence of E2020 by analysis of variance. Wilcoxon's matched-pairs signed-ranks test was used to compare t_{max} for both (R) and (S)-warfarin in the presence and absence of E2020.

Trial Population: Volunteers were non-smoking healthy males aged 18-55 years within 15% of normal weight for height and body build, with no past medical history of clinical significance.

Thirteen volunteers were recruited, of which twelve successfully completed all phases of the study.

All blood pressure and heart rate data are listed in Appendix IV. Table 7 contains the mean group blood pressure at each study timepoint. Samples drawn for warfarin protein binding were not analysed, as there was no evidence to suggest that these would provide any additional useful information.

9.6 Pharmacokinetics

Plasma E2020 concentrations are included in Appendix XII and confirm that concentrations achieved were at steady-state.

9.6.1 (R)-Warfarin

The individual plasma (R)-warfarin concentration-time data are presented in Appendix V in both tabular and graphical format. The Siphar output for the estimation of $t_{½}$ is presented in Appendix VI. Figure 2 shows the mean (± sd) concentration-time data for each treatment. Tables 8 and 9 show the individual pharmacokinetic parameters for treatment A (warfarin plus E2020) and treatment B (warfarin alone), respectively. Mean (± sd) results were:

		Warfarin + E2020	Warfarin Alone
C_{max}	($mg \cdot L^{-1}$)	1.3 (0.3)	1.2 (0.3)
t_{max}	(h) *	1 (0.5-8)	2 (0.5-12)
$AUC_{0-\infty}$	($mg \cdot h \cdot L^{-1}$)	63 (12)	68 (15)
$t_{½}$	(h)	47 (8)	53 (14)
CL/F	($L \cdot h^{-1}$)	0.21 (0.04)	0.19 (0.03)
V_z/F	(L)	14 (2)	14 (3)

* median (range)

9.6.2 (S)-Warfarin

The individual plasma (S)-warfarin concentration-time data are presented in Appendix VII in both tabular and graphical format. The Siphar output for the estimation of $t_{½}$ is presented in Appendix VIII. Figure 3 shows the mean (± sd) concentration-time data for each treatment. Tables 10 and 11 shows the individual pharmacokinetic parameters for treatment A (warfarin plus E2020) and treatment B (warfarin alone), respectively. Mean (± sd) results are:

		Warfarin + E2020	Warfarin Alone
C_{max}	($mg \cdot L^{-1}$)	1.3 (0.3)	1.2 (0.3)
t_{max}	(h) *	1 (0.5-2)	2 (0.5-8)
$AUC_{0-\infty}$	($mg \cdot h \cdot L^{-1}$)	50 (56)	51 (59)
$t_{½}$	(h)	33 (26)	37 (35)
CL/F	($L \cdot h^{-1}$)	0.36 (0.12)	0.35 (0.12)
V_z/F	(L)	13 (3)	14 (2)

* median (range)

Subject 05 exhibited an unusually long $t_{½}$ for S-warfarin leading to the high inter subject variability for $t_{½}$ and $AUC_{0-\infty}$. This occurred in both treatment phases and may be due to differences in metabolism.

9.6.3 Statistical Analysis

Statistical output from SAS are included in Appendix X. The p-values from the ANOVA are summarised below:

p-values from ANOVA

	(R)-warfarin		(S)-warfarin	
	Treatment effects	Period effects	Treatment effects	Period effects
C_{max}	0.2082	0.4485	0.3125	0.4513
$AUC_{0-\infty}$	0.0905	0.9484	0.0885	0.0074
$t_{½}$	0.2269	0.7666	0.1431	0.1508
CL/F	0.0905	0.9484	0.0884	0.0074
V_z/F	0.6329	0.5371	0.2187	0.4946
t_{max} *	0.1649	-	0.0920	-

* Wilcoxon Rank Sums test

There were no statistically significant treatment effects or period effects for any of the pharmacokinetic parameters, except $AUC_{0-\infty}$ for (S)-warfarin, where there was a significant period effect ($p = 0.0074$), however there was no accompanying treatment by period interaction ($p = 0.5755$).

90% confidence intervals were calculated for C_{max} and $AUC_{0-\infty}$ as summarised below:

	(R)-warfarin			(S)-warfarin		
	lower	Mean	upper	lower	Mean	upper
C_{max}	0.97	1.06	1.16	0.95	1.05	1.16
$AUC_{0-\infty}$	0.86	0.92	1.00	0.95	0.98	1.01

9.7 Pharmacodynamics

9.7.1 Prothrombin Time

The individual serial prothrombin time data are presented in Appendix IX in both tabular and graphical format. Figure 4 shows the mean (± sd) prothrombin time vs time data for each treatment. Tables 12 and 13 shows the individual pharmacodynamic parameters for treatment A (warfarin plus E2020) and treatment B (warfarin alone), respectively. Mean (± sd) results were:

		Warfarin + E2020	Warfarin Alone
R_{max}	(s)	30 (9)	29 (8)
t_{max}	(h) *	36 (24-96)	36 (24-72)
AUC_{PT}	(s·h)	3063 (581)	3032 (602)

Subject 05 showed no marked increase in prothrombin time, despite his raised exposure to (S)-warfarin as reflected in $AUC_{0-\infty}$. No baseline sample was obtained for subject 06 for treatment B (warfarin alone). The mean prothrombin time of the other subjects for this treatment was used to calculate AUC_{PT} in this subject.

9.7.2 Statistical Analysis

Statistical output are included in Appendix X. Although there was a statistically significant period effect for R_{max} ($p=0.0055$), there was no statistically significant treatment by period interaction ($p=0.2154$). There was

no statistically significant difference (p=0.3044) between the two treatments for R_{max}.

Although there was a statistically significant period effect for AUC_{PT} (p=0.0009), there was no statistically significant treatment by period interaction (p=0.1196). There was no statistically significant difference (p=0.1118) between the two treatments for AUC_{PT}.

90% confidence intervals were calculated for R_{max} and AUC_{PT} as summarised below:

	Lower	Mean	Upper
R_{max}	0.96	1.02	1.08
AUC_{PT}	0.99	1.01	1.03

10. DISCUSSION

As it can be observed from Figures 2 and 3, the mean profiles for both (R) and (S)-warfarin were superimposable in the presence and absence of E2020. This observation is confirmed by the values for the pharmacokinetic parameters for the two enantiomers (Tables 8 and 9 for (R)-warfarin and tables 10 and 11 for (S)-warfarin) being very similar for the two treatment groups. Similarly prothrombin time profiles for the two treatment groups are also superimposable (Figure 4) and the values for the pharmacodynamic parameters for the two treatment groups are very similar (Tables 12 and 13). The statistical analysis did not show any statistically significant treatment effects for any of the pharmacokinetic or pharmacodynamic parameters. Hence it may be concluded that there is no pharmacokinetic or pharmacodynamic interaction between E2020 and warfarin.

There were 44 adverse events possibly or definitely related to the study drug of which 41 occurred during dosing with E2020. Nausea and vomiting were the most frequently reported adverse event. Other than these gastrointestinal disturbances there

were no specific adverse events to E2020. Warfarin alone appeared to be well tolerated.

In one volunteer a case of neutropenia was recorded. However, this neutropenia occurred 3 weeks after the termination of the E2020 treatment. This dissociation with the study treatment suggests that it is not drug related. A drug-induced neutropenia is known to resolve on withdrawal of the offending drug and in the majority of cases is followed by complete haemopoetic recovery within the next 1-2 weeks. Furthermore, a drug induced neutropenia is usually isolated, without the involvement of the other blood cells (i.e. erythrocytes, platelets). In this subject neutropenia was accompanied with a reduction in the platelet count. The incidence of reduction in neutrophil count and the inconsistent association with administered E2020 treatment, found in this and other volunteers, could possibly be explained by some other causes rather than the E2020 treatment.

11. CONCLUSIONS

- E2020 did not affect the pharmacokinetics or pharmacodynamics of warfarin.

- There were numerous adverse events reported on dosing with E2020, over half of which were nausea and vomiting. Other than these gastrointestinal disturbances there were no specific adverse events to E2020.

TABLE 8 Individual and mean pharmacokinetic parameters of (R)-warfarin following single dose administration of racemic warfarin (25mg) in the presence of multiple dose E2020.

SUBJECT	C_{max} ($mg \cdot L^{-1}$)	t_{max} (h)	$AUC_{0-\infty}$ ($mg \cdot h \cdot L^{-1}$)	$t_{1/2}$ (h)	CL/F ($L \cdot h^{-1}$)	V/F (L)
1						
2						
3						
4						
5						
6						
8						
9						
10						
11						
12						
13						
MEAN	1.3		62.9	47.0	0.21	13.7
MEDIAN		1.0				
SD	0.5		12.5	7.9	0.038	2.3
CV%	24		20	17	19	17
MIN	0.8	0.5	47.9	37.6	0.15	9.9
MAX	1.8	8.0	83.4	65.4	0.26	17.5

TABLE 9 Individual and mean pharmacokinetic parameters of (R)-warfarin following single dose administration of racemic warfarin (25mg) alone.

SUBJECT	C_{max} ($mg \cdot L^{-1}$)	t_{max} (h)	$AUC_{0-\infty}$ ($mg \cdot h \cdot L^{-1}$)	$t_{1/2}$ (h)	CL/F ($L \cdot h^{-1}$)	V/F (L)
1						
2						
3						
4						
5						
6						
8						
9						
10						
11						
12						
13						
MEAN	1.2		68.2	53.1	0.19	14.2
MEDIAN		2.0				
SD	0.3		14.8	14.4	0.035	3.3
CV%	22		22	27	18	23
MIN	0.8	0.5	51.3	35.7	0.12	10.2
MAX	1.6	12.0	104.1	86.8	0.23	21.5

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TABLE 10 Individual and mean pharmacokinetic parameters of (S)-warfarin following single dose administration of racemic warfarin (25mg) in the presence of multiple dose E2020.

SUBJECT	C_{max} ($mg \cdot L^{-1}$)	t_{max} (h)	$AUC_{0-\infty}$ ($mg \cdot h \cdot L^{-1}$)	$t_{1/2}$ (h)	CL/F ($L \cdot h^{-1}$)	V_d/F (L)
1						
2						
3						
4						
5						
6						
8						
9						
10						
11						
12						
13						
MEAN	1.3	1.5	49.7	32.5	0.36	13.3
MEDIAN		1.0				
SD	0.3	0.7	56.2	26.3	0.12	2.6
CV%	27	43	113	81	34	19
MIN	0.8	0.5	25.0	14.3	0.06	9.1
MAX	1.8	2.0	226.8	115.1	0.50	16.6

TABLE 11 Individual and mean pharmacokinetic parameters of (S)-warfarin following single dose administration of racemic warfarin (25mg) alone.

SUBJECT	C_{max} ($mg \cdot L^{-1}$)	t_{max} (h)	$AUC_{0-\infty}$ ($mg \cdot h \cdot L^{-1}$)	$t_{1/2}$ (h)	CL/F ($L \cdot h^{-1}$)	V_d/F (L)
1						
2						
3						
4						
5						
6						
8						
9						
10						
11						
12						
13						
MEAN	1.2	2.7	91.1	36.7	0.35	13.9
MEDIAN		2.0				
SD	0.3	2.2	59.0	35.1	0.119	2
CV%	25	81	115	95	34	17
MIN	0.7	0.5	26.3	17.0	0.055	11
MAX	1.7	8.0	237.1	146.2	0.475	17

TABLE 12 Individual and mean pharmacodynamic parameters of warfarin following single dose administration of racemic warfarin (25mg) in the presence of multiple dose E2020.

SUBJECT	R_{max} (s)	t_{max} (h)	AUC_{PT} (s·h)
1			
2			
3			
4			
5			
6			
8			
9			
10			
11			
12			
13			
MEAN	29.6		3
MEDIAN		36	
SD	8.9		581
CV%	30		19
MIN	19.7	24	2412
MAX	44.5	96	4023

TABLE 13 Individual and mean pharmacodynamic parameters of warfarin following single dose administration of racemic warfarin (25mg) alone.

SUBJECT	R_{max} (s)	t_{max} (h)	AUC_{PT} (s·h)
1			
2			
3			
4			
5			
6			
8			
9			
10			
11			
12			
13			
MEAN	28.7		3032
MEDIAN		36	
SD	8.2		602
CV%	29		20
MIN	17.9	24	2296
MAX	43.0	72	4029

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FIGURE 2 Mean (±sd) plot of R-warfarin concentration time data following single dose administration of racemic warfarin (25mg) to 12 healthy volunteers in the absence and presence of E2020.

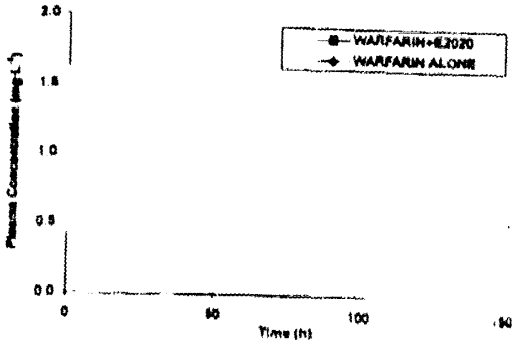


FIGURE 3 Mean (±sd) plot of S-warfarin concentration time data following single dose administration of racemic warfarin (25mg) to 12 healthy volunteers in the absence and presence of E2020.

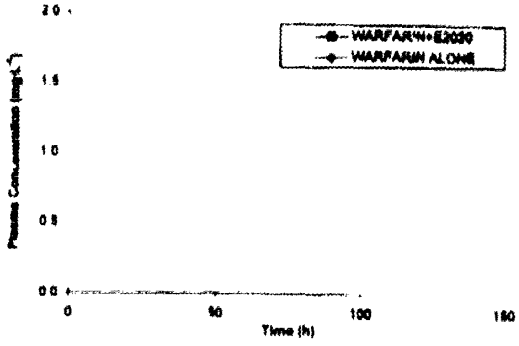
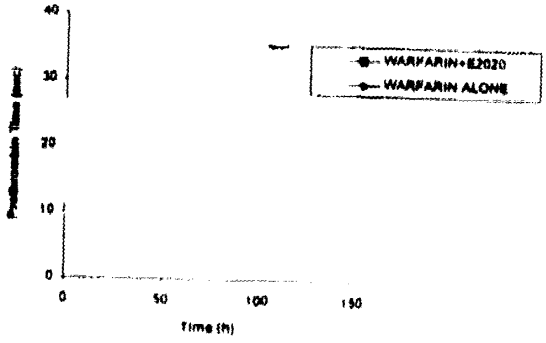


FIGURE 4 Mean (±sd) plot of prothrombin time data following single dose administration of racemic warfarin (25mg) to 12 healthy volunteers in the absence and presence of E2020.



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STUDY 19952677

Protein Binding

Summary

The binding of E2020 *in vitro* to isolated albumin and α_1-acid glycoprotein (α_1-AG) from human, dog and rat serum were investigated by equilibrium dialysis. For all three species, the binding to albumin was higher than that to α_1-AG.

Over a concentration range of 0.5 to 1,000μM, the Scatchard plots for both albumin and α_1-AG were curvilinear. Analyzing these for two types of binding sites gave the parameters tabulated below:

Binding parameters of E2020 to serum proteins

Protein	Kd (μM)		n·Pt	
	Kd_1	Kd_2	n_1·Pt	n_2·Pt
Human albumin	6.81	709	6.48	1300
Human α1-AG	5.20	64.5	0.992	18.2
Dog albumin	12.8	1310	3.18	712
Dog α1-AG	14.7	832	4.39	65.9
Rat albumin	111	2620	23.2	3370
Rat α1-AG	7.34	1680	0.107	76.8

Kd_1 and Kd_2 are the dissociation constants for the first and second binding classes respectively, and n_1·Pt and n_2·Pt are the corresponding binding capacities.

These parameters were used to estimate the total binding of E2020 to albumin and α_1-AG in plasma for unbound concentrations (Cf) ranging from 0.001 to 0.1μM. Results for the total percentage bound were independent of the E2020 concentration in all three species (76-77%, 52-55% and 60-61% for human, dog and rat, respectively). At a Cf for human plasma of 0.01μM (corresponding to a total concentration of 0.0425μM, which is similar to the Cmax at clinically effective doses) the binding to albumin (65.5%) was calculated to be some six times higher than that to α_1-AG(11.1%).

In conclusion: although E2020 is more highly bound to albumin than the α_1-AG of all three species, the percentages bound by the purified proteins from human, dog and rat are all comparatively low (<80%).

Keywords : E2020, protein binding, albumin, α1-acid glycoprotein, binding parameter

4. Results

4.1 Binding of E2020 to Human Albumin and Human α1-AG

The protein binding results for E2020 dialyzed against human albumin and human α1-AG are summarized in Tables 1 and 2, respectively. Over the range of concentrations from 0.5 to 1,000μM, the percentage of E2020 bound to human albumin decreased from 73.9 to 56.1% while that to human α1-AG decreased from 33.5 to 5.5%. Scatchard plots of E2020 binding to both proteins were curvilinear (Figure 3) and two classes of binding sites were identified on human albumin with $n_1 \cdot Pt$ = 6.48 and Kd_1 = 6.81μM, and $n_2 \cdot Pt$ = 1300 and Kd_2 = 709μM; and on human α1-AG with $n_1 \cdot Pt$ = 0.992 and Kd_1 = 5.20μM, and $n_2 \cdot Pt$ = 18.2 and Kd_2 = 64.5μM (Table 7).

These parameters were used to estimate the protein binding in human plasma, using a protein solution containing physiological concentrations of both albumin (40 mg/mL) and α1-AG (1 mg/mL) as a model. As shown in Figure 4, the total percentage bound was 76 - 77%, and was not changed drastically over a range of free E2020 concentrations from 0.001 to 0.1μM. At Cf = 0.01μM (corresponding to Cp = 0.0425μM, which is near the Cmax at clinically effective doses), the percentage of E2020 bound to albumin (65.5%) was higher than that to α1-AG (11.1%).

4.2 Binding of E2020 to Dog Albumin and Dog α1-AG

The protein binding results for E2020 dialyzed against dog albumin and dog α1-AG are summarized in Tables 3 and 4, respectively. Over the range of concentrations from 0.5 to 1,000μM, the percentage of E2020 bound to dog albumin decreased from 44.0 to 30.2% while that to dog α1-AG decreased from 27.9 to 3.8%. Scatchard plots of E2020 binding to both proteins were curvilinear (Figure 3) and two classes of binding sites were identified on dog albumin with $n_1 \cdot Pt$ = 3.18 and Kd_1 = 12.8μM, and $n_2 \cdot Pt$ = 712 and Kd_2 = 1310μM; and on dog α1-AG with $n_1 \cdot Pt$ = 4.39 and Kd_1 = 14.7μM, and $n_2 \cdot Pt$ = 65.9 and Kd_2 = 832μM (Table 7).

These parameters were used to estimate the protein binding in dog plasma, using a protein solution containing physiological concentrations of both albumin (40 mg/mL) and α1-AG (1 mg/mL) as a model. As shown in Figure 5, the total percentage bound was 52 - 55%, and was not changed drastically over a range of free E2020 concentrations from 0.001 to 0.1μM. At

7. Tables and Figures

Table 1 Binding of E2020 to human albumin

E2020 (μM)	Cp (μM)	Cf (μM)	Cb (μM)	Cb/Cf	E2020 bound (%)
0.5	0.402 ± 0.029	0.119	0.256	2.168	68.2
1.0	0.873 ± 0.022	0.227 ± 0.006	0.645 ± 0.017	2.839 ± 0.054	73.9 ± 0.4
2.5	1.99 ± 0.02	0.571 ± 0.013	1.42 ± 0.01	2.482 ± 0.064	71.3 ± 0.5
5.0	3.94 ± 0.02	1.20 ± 0.02	2.74 ± 0.03	2.277 ± 0.065	69.5 ± 0.6
10.0	7.82 ± 0.04	2.54 ± 0.01	5.28 ± 0.04	2.073 ± 0.015	67.4 ± 0.2
25.0	19.5 ± 0.1	6.49 ± 0.06	13.0 ± 0.2	2.003 ± 0.044	66.7 ± 0.5
50.0	38.4 ± 0.1	13.2 ± 0.1	25.3 ± 0.1	1.914 ± 0.028	65.7 ± 0.3
100.0	78.2 ± 0.3	27.1 ± 0.1	51.0 ± 0.3	1.879 ± 0.019	65.3 ± 0.2
250.0	188 ± 1	69.4 ± 0.6	118 ± 1	1.705 ± 0.025	63.0 ± 0.3
500.0	364 ± 2	146 ± 2	217 ± 4	1.487 ± 0.041	59.8 ± 0.7
750.0	548 ± 3	228 ± 2	320 ± 4	1.400 ± 0.030	58.3 ± 0.5
1000.0	711 ± 4	312 ± 4	399 ± 8	1.280 ± 0.043	56.1 ± 0.8

Each value represents the mean ± S.E.M. of three experiments or the mean of two experiments.

Table 2 Binding of E2020 to human α_1-AG

E2020 (μM)	Cp (μM)	Cf (μM)	Cb (μM)	Cb/Cf	E2020 bound (%)
0.5	0.277 ± 0.005	0.194 ± 0.006	0.082 ± 0.010	0.427 ± 0.060	29.7 ± 3.0
1.0	0.533 ± 0.036	0.353 ± 0.022	0.180 ± 0.031	0.515 ± 0.092	33.5 ± 4.2
2.5	1.30 ± 0.01	0.939 ± 0.035	0.36 ± 0.04	0.385 ± 0.051	27.6 ± 2.6
5.0	2.60 ± 0.02	1.98 ± 0.03	0.61 ± 0.05	0.311 ± 0.029	23.6 ± 1.7
10.0	5.24 ± 0.08	4.12 ± 0.16	1.13 ± 0.23	0.279 ± 0.064	21.4 ± 4.1
25.0	13.2 ± 0.0	10.6 ± 0.1	2.6 ± 0.1	0.242 ± 0.012	19.5 ± 0.8
50.0	26.5 ± 0.2	21.9 ± 0.1	4.5 ± 0.1	0.207 ± 0.005	17.2 ± 0.3
100.0	52.5 ± 0.5	44.7 ± 0.5	7.7 ± 0.2	0.173 ± 0.005	14.8 ± 0.3
250.0	128 ± 1	117 ± 1	11 ± 2	0.090 ± 0.016	8.2 ± 1.4
500.0	247 ± 1	234 ± 1	13 ± 1	0.058 ± 0.003	5.5 ± 0.3
750.0	379 ± 1	355 ± 2	24 ± 2	0.068 ± 0.007	6.4 ± 0.6
1000.0	497 ± 2	469 ± 5	28 ± 6	0.060 ± 0.014	5.6 ± 1.3

Each value represents the mean ± S.E.M. of three experiments.

STUDY 19952681

Summary Protein Binding Interaction

Protein binding interactions between E2020 and other drugs (furosemide, digoxin and warfarin) which may be co-administered with E2020 were investigated by using equilibrium dialysis or ultrafiltration.

When E2020 was added at a concentration of 0.3μg/mL to a solution containing human albumin, the percentage of unbound E2020 was 20.8 ± 2.1% and this was not affected by addition of the other drugs. Similarly, at E2020 concentrations of 1.0 and 10μg/mL, the percentages of unbound E2020 were 25.2 ± 0.8% and 28.3 ± 0.2% respectively, and they were not affected by the other drugs.

In addition, the percentage of unbound furosemide was 0.9 ± 0.0% in the absence of E2020 and this was not affected by the addition of E2020 over the range of concentrations from 0.3 to 10μg/mL, the upper limit of which is higher than the C_{max} at the clinical dose. Similarly, the percentages of unbound [^{3}H]-digoxin and [^{14}C]-warfarin were 73.5 ± 0.7% and 0.6 ± 0.0% respectively and were not affected by the addition of E2020.

These interaction studies suggest that E2020 does not affect the binding of furosemide, digoxin, or warfarin to human albumin, and that the binding of E2020 to human albumin is not affected by furosemide, digoxin, or warfarin.

Keywords : E2020, protein binding, human albumin, drug interaction, furosemide, digoxin, warfarin

4. Results and Discussion

In this study, protein binding interactions between E2020 and other drugs (furosemide, digoxin and warfarin) which are predicted to be co-administrated with E2020 were investigated.

The results for the protein binding of E2020 to human albumin in the presence or absence of test drugs are shown in Table 1. When E2020 was added to the protein solution at a concentration of 0.3μg/mL, the percentage of unbound E2020 was 20.8 ± 2.1% and this was not affected by the addition of any drugs. Similarly, at E2020 concentrations of 1.0 and 10μg/mL, the percentages of unbound E2020 were 25.2 ± 0.8% and 28.3 ± 0.2% respectively, and they were not affected by the addition of any drugs.

The results for the protein binding of other drugs to human albumin in the presence or absence of E2020 are shown in Table 2. In the absence of E2020, the percentage of unbound furosemide was 0.9 ± 0.0% and this was not affected by the addition of E2020 over the range of concentrations from 0.3 to 10μg/mL, a range which includes concentrations that are higher than the C_{max} at the clinical dose. Similarly, the percentages of unbound [^{3}H]-digoxin and [^{14}C]-warfarin were 73.5 ± 0.7% and 0.6 ± 0.0% respectively and were not affected by the addition of E2020.

In conclusion, E2020 did not affect the binding of furosemide, digoxin, or warfarin to human albumin, and the binding of E2020 to human albumin was not affected by furosemide, digoxin, or warfarin.

6. Tables and Figures

Table 1 Protein binding of E2020 to human albumin in the presence or absence of other drugs (furosemide, digoxin, and warfarin)

Added E2020 (μg/mL)	Drugs	Bound [a] (%)	Unbound [a] (%)
0.3	Control	79.2 ± 2.2	20.8 ± 2.2
	Furosemide (5μg/mL)	81.4 ± 1.0	18.6 ± 1.0
	Digoxin (2 ng/mL)	80.5 ± 0.6	19.5 ± 0.6
	Warfarin (3μg/mL)	78.9 ± 1.2	21.1 ± 1.2
1.0	Control	74.8 ± 0.8	25.2 ± 0.8
	Furosemide (5μg/mL)	74.9 ± 1.0	25.1 ± 1.0
	Digoxin (2 ng/mL)	73.0 ± 0.3	27.0 ± 0.3
	Warfarin (3μg/mL)	74.7 ± 0.3	25.3 ± 0.3
10.0	Control	71.7 ± 0.2	28.3 ± 0.2
	Furosemide (5μg/mL)	72.2 ± 0.5	27.8 ± 0.5
	Digoxin (2 ng/mL)	72.4 ± 0.1	27.6 ± 0.1
	Warfarin (3μg/mL)	72.9 ± 0.1	27.1 ± 0.1

a)Each value represents the mean ± SEM of three experiments.

Table 2 Binding of other drugs (furosemide, [^{3}H]-digoxin, and [^{14}C]-warfarin) to human albumin in the presence or absence of E2020

Drugs	Added E2020 (μg/mL)	Bound [a] (%)	Unbound [a] (%)
Furosemide (5μg/mL)	0.0	99.1 ± 0.0	0.9 ± 0.0
	0.3	99.2 ± 0.0	0.8 ± 0.0
	1.0	99.1 ± 0.0	0.9 ± 0.0
	10.0	99.1 ± 0.0	0.9 ± 0.0
[^{3}H]-Digoxin (2 ng/mL)	0.0	26.5 ± 0.7	73.5 ± 0.7
	0.3	24.7 ± 1.0	75.3 ± 1.0
	1.0	24.9 ± 0.8	75.1 ± 0.8
	10.0	24.8 ± 1.1	75.2 ± 1.1
[^{14}C]-Warfarin (3μg/mL)	0.0	99.4 ± 0.0	0.6 ± 0.0
	0.3	99.4 ± 0.0	0.6 ± 0.0
	1.0	99.4 ± 0.0	0.6 ± 0.0
	10.0	99.4 ± 0.0	0.6 ± 0.0

a)Each value represents the mean ± SEM of three experiments.

APPENDIX II

Table A6

Assay Methodologies

Study	Type of Biological Fluid	Method	Limit of Quantitation	Relative % Error[a] QC Low (1.5-3.0) ng/mL	Relative % Error[a] QC Medium (7.5-22.5) ng/mL	Relative % Error[a] QC High (35.0-70.0) ng/mL	Precision[b] QC Low (1.5-3.0) ng/mL	Precision[b] QC Medium (7.5-22.5) ng/mL	Precision[b] QC High (35.0-70.0) ng/mL
E2020-A001-001	Plasma			4.8%	3.9%	-2.5%	9.6%	4.9%	6.1%
E2020-A001-002	Plasma			4.8%	3.9%	-2.5%	9.6%	4.9%	6.1%
E2020-A001-003	Plasma			-4.2%	-5.1%	1.2%	7.6%	7.6%	4.1%
E2020-A001-004	Plasma			11.3%	-1.1%	4.9%	8.4%	6.3%	4.8%
E2020-A001-004	Plasma/urine			-	n/a	-	-	n/a	-
E2020-A001-005	Plasma			-	5.6%	-	6.6%	3.5%	-
E2020-A001-006	Plasma			7.0%	0.4%	2.6%	5.9%	5.8%	4.3%
E2020-A001-007	Plasma			-1.5%	-7.9%	-1.1%	6.1%	3.2%	2.9%
E2020-A001-008	Plasma			10.0%	4.1%	1.4%	6.4%	4.2%	3.2%
E2020-A001-009	Plasma			1.7%	3.6%	-	23.9%	5.0%	-

[a]Between run relative percent error (i
[b]Between run coefficient of variation (interday).

CONFIDENTIAL

Table A6 (Cont'd)

Assay Methodologies

Study	Type of Biological Fluid	Method	Limit of Quantitation	Relative % Error[a] QC Low (1.5-3.0) ng/mL	QC Medium (7.5-22.5) ng/mL	QC High (35.0-70.0) ng/mL	Precision[b] QC Low (1.5-3.0) ng/mL	QC Medium (7.5-22.5) ng/mL	QC High (35.0-70.0) ng/mL
E2020-A001-010	Plasma			-1.3%	4.4%	-	6.4%	5.9%	-
E2020-A001-011	Plasma			-2.7%	-0.6%	6.2%	7.7%	4.2%	4.5%
E2020-A001-301	Plasma			4.3%	-2.3%	1.7%	9.9%	6.6%	4.6%
E2020-A001-302	Plasma			8.0%	6.7%	5.8%	6.2%	6.9%	5.2%
E2020-E044-001	Plasma			-0.2%	-1.0%	0.7%	6.5%	2.8%	2.5%
E2020-J081-002	Plasma			-	-	-	-	3.5%	-

[a]Between run relative percent error (in accuracy).
[b]Between run coefficient of variation (precision).

APPENDIX III

Kawashima

Master Production Record and Batch Production Record of Drug Products
Pharmaceutical Development Research Laboratories, Eisai Co., Ltd.

Product: BNAG- FT 5mg	Dosage form: Film-coated tablet
MPR No.: MPR-BNAG-FT-5-BS-0038-00	Manufacturing site: Manufacturing area in solid Formulation lab

Lot No.: K46016 22

Composition of BNAG-FT5mg

Ingredient (Name authorized in-house)	Code No.	Purpose	Amount/tablet	Charge-in amount

a) Batch size
b) Batch size

c) Batch size

Key (Name authorized in-house vs. name given official compendium)

9 Pages
Purged

APPENDIX IV

4 Pages
Purged

APPENDIX V

14 Pages

Purged

List of Additional studies that were not reviewed.

1) A placebo controlled multiple-dose study to evaluate the safety and tolerance and to establish the pharmacokinetic profile of E2020 in healthy male subjects (Study E2020-Jo81-003).

2) An Ascending, single-dose, safety and tolerance study of an oral formulation of a cholinesterase inhibitor, E2020, in healthy male volunteers (Study E2020-A001-001).

3) A single and multiple-dose study of the central and peripheral pharmacokinetics and pharmacodynamics of E2020 in healthy male and female volunteers (Study E2020-A001-003).

4) A single ascending oral dose study to evaluate the safety, tolerance and pharmacokinetic profile of E2020 at dose levels of 0.3, 1.0, 2.0, 5.0, 8.0, and 10 mg in Japanese healthy subjects (Study E2020-J081-001).

AUG 21 1996

OCTIRAI

AUG 26 1996

OFFICE OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

Donepezil Hydrochloride (E2020; Aricept®)
5 and 10 mg Tablets
NDA 20-690

Eisai America Inc.,
300 Frank W. Burr Blvd.
Teaneck, NJ 07666-6741

Reviewer: Vijay K. Taminara, Ph.D.
Indication: Alzheimer's Dementia
Classification: 1P
Type of Submission: Response to Comments

Submission Date:
August 2, 1996

In the present submission, the sponsor has provided responses to the comments of the reviewer communicated in a telecon on July 24, 1996 (Attachment 1). The responses provided by the sponsor are satisfactory and are acceptable. These responses also address Comments 5-9 of the Original Office of Clinical Pharmacology and Biopharmaceutics (OCPB) NDA review dated August 1, 1996. The details of these Comments are as follows:

Comment 5: In the bioequivalence studies, 90% confidence interval analysis should be performed using log transformed data for $AUC_{0-\infty}$, instead of using only untransformed data.

The sponsor performed 90% confidence interval analysis using log transformed data for $AUC_{0-\infty}$ (Attachment 2) for both the pivotal bioequivalency studies (Studies E2020-A001-009 and 010). From the results, it was observed that the to-be-marketed film coated tablets are also bioequivalent to the clinical lot based on log transformed $AUC_{0-\infty}$.

Comment 6: The sponsor is requested to submit the study reports of the population pharmacokinetic analysis including the control files to evaluate the effect of population covariates on the pharmacokinetics of E2020.

The sponsor has provided the study report and the control files of the population pharmacokinetic analysis to evaluate the effect of population covariates on the pharmacokinetics of E2020. However, results of the NONMEM analyses were not used in support of any conclusions related to E2020 pharmacokinetics or pharmacodynamics.

Comment 7: The sponsor is requested to submit the composition of the formulations used in biostudies (Kawashima, Lot # 46016ZZZ, 41016ZZA, Lot # 95025C; Pfizer Inc., Lot # N5111) and at least three representative lots from clinical trials 301 (e.g., Lot #s: K3Y002ZZZ, K3X015ZZZ, and K42009ZZZ) and 302 (e.g., Lot #s: K3Y003ZZZ, K37017ZZA, and K42016ZZZ).

The sponsor has provided the composition of formulations used in biostudies as well as in clinical trials (Attachment 3). The formulation compositions of the different lots used in the clinical trials and the different clinical lots used in the biostudies were found to be identical.

Comment 8: The sponsor is requested to submit dissolution profiles, both graphical as well as tabular data of 12 individual tablets and mean data, for each strength of Aricept tablets manufactured at both the sites
Pfizer Inc., Brooklyn, NY) in 3 media
This will provide the necessary dissolution data to support the biowaiver request for the 10 mg tablet and also allow for setting a more appropriate dissolution specification for both the 5 and 10 mg tablets.

The sponsor provided dissolution profiles of 12 individual tablets for each strength of Aricept tablets manufactured at both sites
and Pfizer Inc., Brooklyn, NY) in 3 media .....
From the results it was observed that the 5 and 10 mg strength tablets have comparable dissolution profiles. Based on the dissolution data, compositional proportionality of the 5 and 10 mg tablets, linear pharmacokinetics over the proposed dosage regimen; and high solubility and high permeability of E2020, biowaiver can be granted for the 10 mg tablet.

Comment 9: The sponsor is requested to adopt the following "interim" dissolution methodology and specification for Aricept 5 mg film-coated tablet:

Medium:
Apparatus:
Specification:

Since the sponsor has provided the dissolution profiles of both the 5 and 10 mg tablets in 3 media and also satisfied the biowaiver criteria, the sponsor is requested to adopt the following as the "final" dissolution methodology and specification for Aricept 5 and 10 mg film-coated tablets:

Medium:
Apparatus:
Specification:

****

In addition, the sponsor has provided corrected table of dissolution summary and corrected synopsis of bioequivalence study reports to reflect the correct lot numbers of tablets used in these studies (Attachment 5).

Recommendation: The responses provided by the sponsor are satisfactory and found to be acceptable. These responses also address earlier Comments 5-9 of the Original OCPB review dated August 1, 1996. Further, the sponsor is requested to adopt the dissolution methodology and specification for Aricept 5 and 10 mg film-coated tablets as outlined in Comment 9. Please forward this Recommendation to the sponsor.

08/21/96

Vijay K. Tammara, Ph. D.
Division of Pharmaceutical Evaluation I

First Draft Prepared on August 20, 1996
RD Initialed by M. Hossain, Ph. D. On August 21, 1996

FT Initialed by M. Hossain, Ph. D. M Hossain 8/21/96

CC: NDA 20,690 (orig.), HFD-120, HFD-860 (Tammara, Hossain, Malinowski), HFD-340 (Vish), HFD-019, HFD-870 (Clarence Bott (Rm 13B31 PKLN): Drug, Chron, Division, and Reviewer Files).

ATTACHMENT 1

Eisai America, Inc.

GLENPOINTE CENTRE EAST
300 FRANK W BURR BLVD
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PHONE: (201) 692-9160
FAX: (201) 692-9183

August 2, 1996

Paul Leber, M.D.
Director
Division of Neuropharmacologic Drug Products
4th Floor
Office of Drug Evaluation
Food and Drug Administration
Attention: Document Control Room
Woodmont #2 Building
1451 Rockville Pike
Rockville, MD 20852

SUBJECT: NDA 20,690 - ARICEPT ™ (donepezil hydrochloride) Tablets
RESPONSE TO FDA REQUEST FOR ADDITIONAL INFORMATION

Dear Dr. Leber:

On July 24, 1996, a teleconference was held between representatives from the FDA and Eisai to discuss several questions raised by the FDA regarding product formulation, lot numbers used in the Pharmacokinetic Phase I studies and other Pharmacokinetic information. The following individuals were present:

Eisai America, Inc.
Dr. N.J. Farina, Senior Directo., Regulatory Affairs
M. Parsi, Manager, Regulatory Affairs

FDA, Division of Biopharmaceutics
Dr. M. Hossain, Team Leader
Dr. V. Tammara, Pharmacokinetic Reviewer

Our resp onse to the issues discussed is as follows:

1. **Please provide a full statement of composition for the tablets used in Bioequivalence Studies E2020-A001-009 and E2020-A001-010.**

   In study # E2020-A001-009, Lot 95025C manufactured by was compared to the reference Lot K46016ZZZ produced at Eisai (Japan).

Further, in study # E2020-A001-010, Lot N5111 manufactured by Pfizer (Brooklyn, US) was compared to the reference Lot K41016ZZA produced at Eisai (Japan).

The compositions of the . Pfizer tablets are essentially the same as that of the reference lots manufactured at Eisai, except for the following minor changes:

(i) Excipient specifications (
(ii) Eisai tablets were polished with a trace amount c mg/tablet), which was deemed unnecessary upon further progress in the product development. Therefore, the proposed marketing formulation does not contain

In addition, Pfizer's 5 mg tablets were film-coated usin This preparation is a customer specified, identical dry formulation of the standard Eisai film-coating materials. Th is a mixture of the dry powders manufactured by ready for reconstitution in water.

It should be noted that these changes did not impact bioequivalence, as demonstrated in the biostudies E2020-A001-009 and E2020-A001-010.

The quantitative compositions of the above referenced 5 mg lots are enclosed. [redacted]

2. **Please provide the tablet formulation for the lots used in the clinical studies E2020-A001-301 and E2020-A001-302.**

The tablet composition of the clinical trial lots is identical to that of Eisai's film-coated tablets used in the bioequivalence studies (i.e. lots K46016ZZZ and K41016ZZA).

Appended [redacted] is the quantitative composition of the lots used in the Phase III clinical trials.

3. **Please provide the formulations of the 5 mg and 10 mg strength tablets proposed for marketing by both manufacturing sites and Pfizer. Also describe the differences between the formulations and manufacturing procedures used at each site.**

The compositions of the 5 mg tablets are the same as those used in the bioequivalence studies.

The 10 mg tablets also contain in the film-coating, so that they would easily be distinguished from the 5 mg strength.

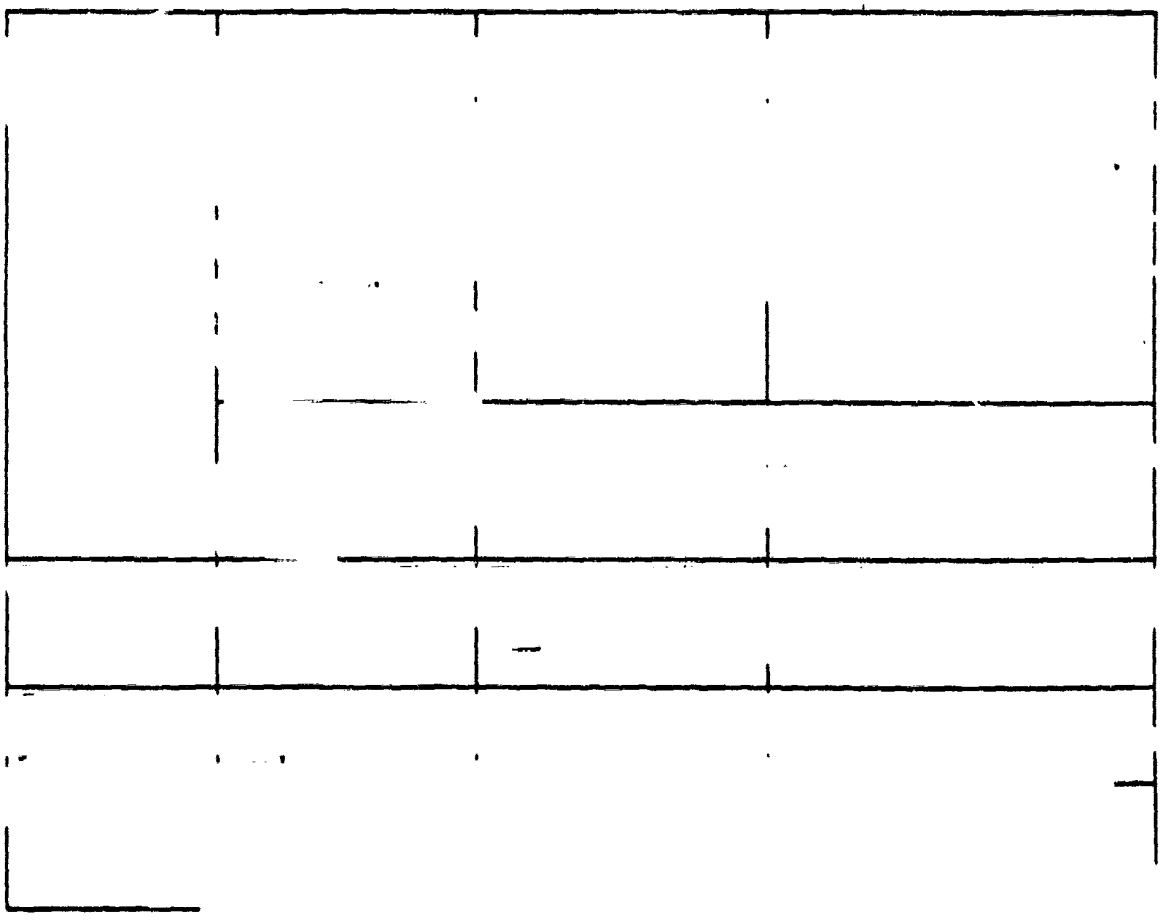
Pfizer's 10 mg tablets use , which as previously indicated is a dry powder mix reconstituted in water.

Please note that as described under Item 1, the only difference between the formulations of Pfizer and is the use of by Pfizer, during the film-coating process.

The compositions of the proposed commercial 5 mg and 10 mg tablets for both and Pfizer are included [redacted]

With regards to the manufacturing procedures employed at the two sites, there are some minor differences such as the use of different equipment in the granulation, blending and tabletting stages. It should be noted that these pieces of equipment essentially function in the same manner, and the in-process controls utilized at each facility are basically the same.

The following summary table describes these differences in further detail:



4. **Please provide dissolution profile data for the 5 mg (biobatch) and 10 mg drug product manufactured at both and Pfizer facilities. The data generated should be for 12 tablets of each strength tested in three different media such as, deionized water,**

In addition, please provide any available dissolution profile data on the biobatches and the Phase III clinical lots manufactured at Eisai, Japan.

[redacted] this response, encompasses 12 tablet dissolution profile data generated on the following lots using the three media referenced:

- 5 mg biobatch #95025C
- 10 mg NDA stability batch # 95028C

Pfizer
- 5 mg biobatch # N5111
- 10 mg NDA stability batch # N5110

Additionally, included in this attachment are various tables summarizing all the dissolution testing information available for the bioequivalency study reference batches (K46016ZZZ and K41016ZZA), as well as the lots used in clinical studies E2020-A001-301 and E2020-A001-302.

5. **There are some inconsistencies between the lot numbers indicated in the Table A3 (Table of Pharmacokinetic Studies), Table A5 and the Phase I study reports. Please correct the documents as needed.**

As requested, the discrepancies with regards to the lot numbers have been corrected in the Tables A3 and A5 (presented in the NDA Volume 1.049), and the relevant study reports.

These discrepancies have occurred due to typographical or transcription errors. We apologize for the inconvenience this may have caused during the course of your review.

Appended [redacted] are the updated Table A5, and excerpts from Table A3 and the study reports corrected to reflect the correct lot numbers.

6. **For the Bioequivalence Studies E2020-A001-009 and E2020-A001-010, please calculate the log transform data using AUC $_{0-\infty}$. The NDA encompasses calculations using AUC $_{0-168}$ and untransform data using AUC $_{0-\infty}$.**

   Table 8 comparing pharmacokinetic parameters for vs. Kawashima (Eisai) and Pfizer vs. Kawashima, as well as the Statistical Appendix VII have been updated to include the additional analyses requested.

   The $\log_{10}$ AUC $_{0-\infty}$ is presented on the first page of Table 8, for each study. As with the other pharmacokinetic parameters, bioequivalence was observed between the reference and or Pfizer tablets in both biostudies.

   The updated Table 8 and Statistical Appendix VII are enclosed [redacted].

7. **The pharmacokinetic calculations of the Phase I Study # E2020-A001-003 were carried out using geometric means. Please perform these calculations using arithmetic means and standard deviations. The pharmacokinetic parameters should be listed by individual as well as mean values.**

   Tables containing the individual and arithmetic mean pharmacokinetic parameters for Study E2020-A001-003, are provided [redacted].

8. **When performing the analyses for Studies E2020-A001-301 and E2020-A001-302, what type of model and control files were used?**

   Enclosed [redacted] are three 3.5 inch diskettes which contain examples of data and models used to analyze the relationship between E2020 (donepezil hydrochloride) plasma concentrations and the inhibition of acetylcholinesterase (AChE), for the referenced studies.

   A description of the specific files included in each diskette is also provided therein.

We hope that the above has addressed your comments sufficiently. If there are any further questions, please do not hesitate to contact us at any time.

Please be advised that material and data contained in this submission are confidential. The legal protection of such confidential material is hereby claimed under applicable provisions of 18 U.S.C., Section 1905 or 21 U.S.C., Section 331 (J).

Sincerely,

Nicholas J. Farina, Ph.D.
Senior Director, Regulatory Affairs

NJF/gg
Encl.
Desk Copy to Dr. V. Tammara

ATTACHMENT 2

Study E2020-A001-009
Table 8 (A Comparison of Pharmacokinetic Parameters) and Appendix VII

(NDA Volumes 1.075 and 1.076)

Eisai America, Inc.
Protocol: E2020-A001-009
Investigator:

Table 8
A Comparison of Pharmacokinetic Parameters

	E2020 5mg	E2020 5mg	KAWASHIMA® E2020 5mg	90% C.I. for Ratio* Test/Reference	Two One-sided@ t-tests (T_U, T_L)	90% C.I. for Ratio* Test/Reference	Two One-sided@ t-tests (T_U, T_L)
$AUC_{0\text{-}168\ hrs.}$ (ng*hr/mL)							
N	18	18	18				
Mean (S.E.)	340.7 (24.7)	324.2 (19.2)	323.6 (19.4)	(100.8 , 109.7)	(-5.59 , 9.60)	(95.7 , 104.6)	(-7.52 , 7.67)
Min - Max	122.4 - 489.8	181.6 - 456.6	176.8 - 482.2				
Coeff. Var.	18.6%	11.1%	11.2%				
$Log_{10} AUC_{0\text{-}168\ hrs.}$ (ng*hr/mL)							
N	18	18	18				
Mean (S.E.)	2.505 (.037)	2.497 (.027)	2.496 (.028)	(98.5 , 107.8)	(-7.28 , 9.55)	(95.9 , 104.9)	(-8.29 , 8.54)
Min - Max	2.088 - 2.690	2.259 - 2.660	2.247 - 2.683				
Coeff. Var.	0.074%	0.074%	0.074%				
Geometric Mean	322.7	314.1	313.1				
$AUC_{0-\infty}$ (ng*hr/mL)							
N	18	18	18				
Mean (S.E.)	454.9 (34.7)	452.0 (31.1)	437.4 (29.8)	(98.6 , 109.4)	(-4.99 , 7.48)	(97.9 , 108.8)	(-5.19 , 7.28)
Min - Max	167.2 - 769.7	279.1 - 741.7	249.8 - 759.8				
Coeff. Var.	13.1%	13.2%	13.6%				
$Log_{10} AUC_{0-\infty}$ (ng*hr/mL)							
N	18	18	18				
Mean (S.E.)	2.634 (.037)	2.639 (.029)	2.624 (.030)	(97.2 , 107.6)	(-6.69 , 8.20)	(98.3 , 108.8)	(-6.32 , 8.57)
Min - Max	2.223 - 2.886	2.466 - 2.870	2.398 - 2.881				
Coeff. Var.	0.084%	0.084%	0.084%				
Geometric Mean	430.2	435.8	420.6				

Data Source: Statistical Appendix VII. *R = Kawashima is the reference formulation.* *continued...*

** 90% confidence interval on the ratio of test formulation to the reference formulation.*

@ Schuirmann's Two one-sided tests:

Formulations are deemed bioequivalent if the classical (shortest) 90% Confidence Interval is within the 80-120% interval for the raw data, and within the 80-125% interval for the log-transformed data. Bioequivalence is also declared if the absolute value of both t-tests are greater than 1.694

Eisai America, Inc.
Protocol: E2020-A001-009
Investigator:

Table 8
A Comparison of Pharmacokinetic Parameters

	E2020 5mg	E2020 5mg	KAWASHIMA[R] E2020 5mg	90% C.I. for Ratio* Test/Reference	Two One-sided@ t-tests (T_U, T_L)	90% C.I. for Ratio* Test/Reference	Two One-sided@ t-tests (T_U, T_L)
C_{max} (ng/mL)							
N	18	18	18				
Mean (S.E.)	7.5 (0.3)	7.3 3)	7.4 (0.2)	(97.7 , 106.2)	(-7.16 , 8.68)	(94.2 , 102.7)	(-8.52 , 7.30)
Min - Max	5.7 - 10.0	5.4 10.0	5.6 - 8.6				
Coeff. Var.	10.5%	10.3%	10.7%				
$Log_{10}C_{max}$ (ng/mL)							
N	18	18	18				
Mean (S.E.)	0.871 (.017)	0.856 (.017)	0.866 (.012)	(97.1 , 105.7)	(-8.43 , 9.47)	(93.7 , 102.8)	(-9.85 , 8.05)
Min - Max	0.758 - 1.002	0.734 - 1.001	0.749 - 0.933				
Coeff. Var.	0.070%	0.070%	0.070%				
Geometric Mean	7.4	7.2	7.3				
t_{max} (Hour)							
N	18	18	18				
Mean (S.E.)	4.2 (0.3)	4.4 (0.4)	4.2 (0.2)	(87.7 , 112.3)	(-2.75 , 2.75)	(94.4 , 119.0)	(-1.83 , 3.66)
Min - Max	2.0 - 6.0	3.0 - 8.0	3.0 - 6.0				
Coeff. Var.	30.9%	29.0%	30.9%				
$t_{1/2}$ (Hour)							
N	18	18	18				
Mean (S.E.)	63.23 (5.45)	65.64 (4.26)	61.75 (4.58)	(95.5 , 109.3)	(-4.30 , 5.47)	(99.4 , 113.2)	(-3.34 , 6.42)
Min - Max	26.31 - 116.3	39.62 - 96.99	34.19 - 111.2				
Coeff. Var.	17.0%	16.4%	17.4%				

Data Source: Statistical Appendix VII. *R = Kawashima is the reference formulation.*

** 90% confidence interval on the ratio of test formulation to the reference formulation.*

● Schuirmann's Two one-sided tests:
Formulations are deemed bioequivalent if the classical (shortest) 90% Confidence Interval is within the 80-120% interval for the raw data, and within the 80-125% interval for the log-transformed data. Bioequivalance is also declared if the absolute value of both t-tests are greater then 1.694

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Study E2020-A001-010
Table 8 (A Comparison of Pharmacokinetic Parameters) and Appendix VII

(NDA Volumes 1.077 and 1.078)

Eisai America, Inc.
Protocol: E2020-A001-010
Investigato

Table 8
A Comparison of Pharmacokinetic Parameters

	PFIZER E2020 5mg	KAWASHIMA[R] E2020 5mg	PFIZER vs. KAWASHIMA[R] 90% C.I. for Ratio* Test/Reference	PFIZER vs. KAWASHIMA[R] Two One-sided@ t-tests (T_U, T_L)
$AUC_{0\text{-}168\ hrs.}$ (ng*hr/mL)				
N	12	12		
Mean (S.E.)	349.0 (31.1)	358.2 (30.0)	(94.8 , 100.0)	(-15.13 , 11.66)
Min - Max	195.3 - 495.3	188.5 - 499.5		
Coeff. Var.	5.3%	5.2%		
$Log_{10}AUC_{0\text{-}168\ hrs.}$ (ng*hr/mL)				
N	12	12		
Mean (S.E.)	2.521 (.043)	2.535 (.041)	(93.9 , 100.0)	(-13.91 , 10.46)
Min - Max	2.291 - 2.695	2.275 - 2.699		
Coeff. Var.	0.042%	0.042%		
Geometric Mean	331.9	342.6		
$AUC_{0-\infty}$ (ng*hr/mL)				
N	12	12		
Mean (S.E.)	487.1 (34.5)	488.9 (36.7)	(99.0 , 103.6)	(-13.93 , 15.86)
Min - Max	314.8 - 711.6	259.9 - 707.9		
Coeff. Var.	4.6%	4.7%		
$Log_{10}AUC_{0-\infty}$ (ng*hr/mL)				
N	12	12		
Mean (S.E.)	2.675 (.032)	2.666 (.036)	(99.0 , 105.2)	(-11.33 , 13.59)
Min - Max	2.498 - 2.852	2.415 - 2.850		
Coeff. Var.	0.041%	0.041%		
Geometric Mean	473.3	463.8		

Data Source: Statistical Appendix VII. *R = Kawashima is the reference formulation.*

** 90% confidence interval on the ratio of test formulation to the reference formulation.*

@ Schuirmann's Two one-sided tests:

Formulations are deemed bioequivalent if the classical (shortest) 90% Confidence Interval is within the 80-120% interval for the raw data, and within the 80-125% interval for the log-transformed data.

Bioequivalance is also declared if the absolute value of both t-tests are greater then 1.717

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Eisai America, Inc.
Protocol: E2020-A001-010
Investigat

Table 8
A Comparison of Pharmacokinetic Parameters

	PFIZER E2020 5mg	KAWASHIMA[R] E2020 5mg	PFIZER vs. KAWASHIMA[R] 90% C.I. for Ratio* Test/Reference	PFIZER vs. KAWASHIMA[R] Two One-sided● t-tests (T_U, T_L)
C_{max} (ng/mL)				
N	12	12		
Mean (S.E.)	6.8 (0.3)	6.9 (0.3)	(93.8 , 102.0)	(-9.24 , 7.50)
Min - Max	5.0 - 8.0	5.0 - 8.8		
Coeff. Var.	8.5%	8.3%		
$Log_{10}C_{max}$ (ng/mL)				
N	12	12		
Mean (S.E.)	0.829 (.019)	0.837 (.019)	(94.0 , 102.1)	(-10.10 , 8.43)
Min - Max	0.698 - 0.982	0.699 - 0.944		
Coeff. Var.	0.055%	0.055%		
Geometric Mean	6.7	6.9		
t_{max} (Hour)				
N	12	12		
Mean (S.E.)	3.9 (0.3)	4.1 (0.3)	(83.1 , 108.7)	(-3.22 , 2.13)
Min - Max	2.0 - 6.0	2.0 - 6.0		
Coeff. Var.	27.0%	25.9%		
$t_{1/2}$ (Hour)				
N	12	12		
Mean (S.E.)	77.72 (3.56)	70.86 (4.20)	(103.9 , 115.5)	(-3.05 , 8.78)
Min - Max	57.89 - 97.60	37.77 - 90.85		
Coeff. Var.	10.7%	11.7%		

Data Source: Statistical Appendix VII.
R = Kawashima is the reference formulation.
** 90% confidence interval on the ratio of test formulation to the reference formulation.*
● Schuirmann's Two one-sided tests:
Formulations are deemed bioequivalent if the classical (shortest) 90% Confidence Interval is within the 80-120% interval for the raw data, and within the 80-125% interval for the log-transformed data. Bioequivalance is also declared if the absolute value of both t-tests are greater then 1.694

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ATTACHMENT 3

25 Pages
Purged

Corrected Excerpts From Study Report E2020-A001-010

(NDA Volume 1.077)

CLINICAL STUDY SYNOPSIS

Title: A Study of the Bioequivalence of Two Tablet Formulations of E2020, in Healthy Volunteers; Study #: E2020-A001-010

Investigator:

Objective: The objective of this study was to determine the bioequivalence of a new 5-mg E2020 tablet formulation relative to the E2020 reference 5-mg tablet formulation used in all clinical trials.

Study Design: This study was a randomized, single-dose, open label, two-period crossover study which was conducted at a single investigational site. Each treatment period was followed by a 2-week washout period.

Study Population: Participants in this study were healthy, ambulatory, non-smoking male volunteers, aged 18-45 years. A total of 12 volunteers were randomized into this study and all 12 completed the study without incident. All participants were Caucasion, and ranged in age from 19 to 44 years and in weight from 68.5 to 86.0 kg.

Treatment Groups: Subjects randomly received each of the two following E2020 formulations:

Formulation A: 5.0-mg, film-coated E2020 tablets produced by Eisai Company, Ltd. in Kawashima, Japan. This is the REFERENCE formulation. Lot # K41016ZZA

Formulation D: 5.0 mg, film-coated tablets produced by Pfizer Inc., Brooklyn, New York, USA. This is the TEST formulation. Lot # N5111

Medication was administered after a fast of at least eight hours and was accompanied by a total of 250-ml of tap water.

Methods *(Clinical)*: Volunteers were screened by medical history, ECG, and laboratory and physical examinations $\leq$ 2 weeks prior to the start of the study. For each Treatment period, subjects were admitted to the study site on the evening of day 0, at least 12 hours prior to drug administration. Upon admission, a routine physical examination was conducted. Entry vital signs (sitting position), and weight were recorded, and urine specimens for drug use/abuse screening were collected. A light snack was served 10 hours prior to drug administration, after which an absolute fast, from both food and fluids (except water), was maintained. The following morning, prior to drug administration, clinical laboratory and analytical samples were collected. During each

4.0 MATERIALS AND METHODS

4.1 DRUGS AND DOSAGES

4.1.1 Description of Test drugs

Test drugs are defined as any medications given as part of the study protocol. For this study, the test drugs were two different 5-mg formulations of E2020.

The following is a brief description of the E2020 formulations used in this study:

Formulation A: , film-coated tablets containing
(Reference formulation) 5.0-mg of E2020 hydrochloride.

Manufactured by Eisai Company, Ltd., JAPAN.
Manufacturing date: July 12, 1994.
Batch size:
Lot Number: K41016ZZA.

This formulation was produced at the Eisai manufacturing facility in Kawashima, Japan. It contains and was used in all clinical trials conducted in the USA.

Formulation D: film-coated tablets containing
(Test Formulation) 5.0-mg of E2020 hydrochloride.

Manufactured by Pfizer Inc., USA.
Manufacturing date: June, 1995
Batch size: 10% of commercial lot.
Lot Number: N5111

This formulation was produced at the Pfizer manufacturing facility in Brooklyn, New York. These tablets are from a 1/10 stability batch and do not contain

4.1.2 Rationale for Dosage Selection

The dose evaluated was selected based upon clinical efficacy studies of E2020 conducted in the United States and was within the range known to have clinical utility.

4.1.3 Shipment of Drug Supplies

Drug supplies were sent to the Clinical Investigator only when the following documentation had been received by Eisai America, Inc.:

Corrected Excerpt From Study Report E2020-A001-011

(NDA Volume 1.079)

4.0 MATERIALS AND METHODS

4.1 DRUGS AND DOSAGES

4.1.1 Description of Test drugs

Test drugs are defined as any medications given as part of the study protocol. For this study, the test drugs were E2020 and placebo.

The following is a brief description of the formulations used in this study:

E2020: film-coated tablets containing 5.0-mg of E2020 hydrochloride.

Manufactured by Eisai Company, Ltd., JAPAN.
Manufacturing date: December 10, 1993.
Batch size:
Lot Number: K49007ZZA

Placebo: film-coated placebo tablets.

Manufactured by Eisai Company, Ltd., JAPAN.
Manufacturing date: November 26, 1993.
Batch size
Lot Number: K3X010ZZZ

The doses of E2020 evaluated in this study were 5-mg and 10-mg. To minimize reactions to acute, extensive inhibition of cholinesterase, the 10-mg dose was initiated using a scheduled titration scheme. The subjects received 5-mg doses of E2020 for the first 7 days, after which the 10-mg dose was administered for the remaining 21 days.

4.1.2 Rationale for Dosage Selection

The doses evaluated were selected based upon clinical efficacy studies of E2020 conducted in the United States and were within the range known to have clinical utility.

4.1.3 Shipment of Drug Supplies

Drug supplies were sent to the Clinical Investigator only when the following documentation had been received by Eisai America, Inc.:

1. Written proof of approval of both the protocol and its consent form by the Institutional Review Board of the institution where the study was to be conducted.

2. A copy of the certification and a table of the normal ranges for the reference laboratory conducting the clinical laboratory tests required by this protocol.

PHARMACOLOGY

NDA 20-690 Aricept (donepezil HCl) Tablets

Classification: 1P

Date	Document	Reviewer
08/07/96	Team Leader Memo	Glenna Fitzgerald, Ph.D.
06/06/96	NDA Review	Barry Rosloff Ph.D.

Pharmacologist Review

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESLARCH

DATE: August 7, 1996

TO: NDA 20-690
Donepezil HCl; Aricept
Esai America, Inc.

FROM: Glenna G. Fitzgerald, Ph.D GGF
Pharmacology Team Leader
Division of Neuropharmacological Drug products, HFD-120

SUBJECT: Approvability for Alzheimer's Disease

The pharmacology and toxicology studies submitted to this NDA for Aricept support its approval as therapy for an indication of Alzheimer's disease. These studies are reviewed and summarized in the extensive review by Dr. Barry Rosloff. Only one issue remains outstanding. As has been usual Division policy for drugs in this therapeutic category, the sponsor was granted permission to complete two lifetime carcinogenicity studies post-approval. The study protocols (mice and rats) were reviewed by the CAC-EC on 3/22/94, and recommendations transmitted to the sponsor on 4/7/94. The studies are currently underway and the reports of those studies should be available within the next 6 months. The sponsor should be reminded of this commitment in the approvable letter, and requested to submit the study reports in a timely fashion.

NDA 20-690
HFD-120
Leber
Levin
Fitzgerald
Rosloff
Higgins

Barry N. Rosloff, Ph.D.
6/6/96

Pharmacologist Review of NDA 20-690
Original Summary

SPONSOR: Eisai America, Inc.
Glenpointe Centre East
300 Frank W. Burr Blvd.
Teaneck, New Jersey 07666-6741

DRUG: E 2020
(donepezil, Aricept®)

(See attached page for structure and chemical names)

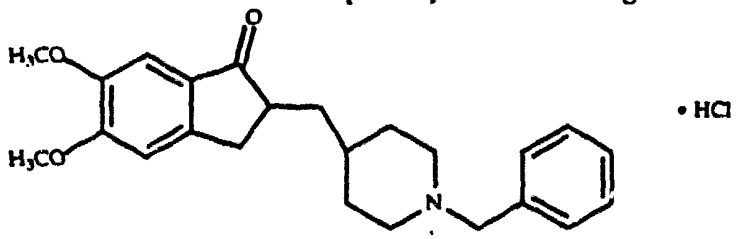
CATEGORY: Alzheimer's disease
(acetylcholinesterase inhibitor)

RELATED IND: INL

2. Nomenclature/Formulae

2.1 Structural Formula

The structural formula of donepezil hydrochloride is given below:



2.2 Molecular Formula and Molecular Weight

Donepezil hydrochloride has the formula $C_{24}H_{29}NO_3 \cdot HCl$.

Donepezil hydrochloride has the molecular weight 415.96.

2.3 Nomenclature

U.S.A.N.: donepezil hydrochloride

International Non-proprietary Name (I.N.N.): donepezil

Chemical Names:

1. (±)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one, hydrochloride (USAN and CAS)

2. 2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one, hydrochloride (CAS)

3. (±)-2-[(1-benzyl-4-piperidyl)methyl]-5,6-dimethoxy-1-indanone, hydrochloride (USAN)

4. (*RS*)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methylpiperidine, hydrochloride (IUPAC)

Chemical Abstracts Services Numbers: [142057-77-0] and [120011-70-3]

Proprietary or Trade Names: ARICEPT ™

Other Names: E2020, BNAG

2.4 Laboratory Code

Donepezil hydrochloride has been assigned the laboratory code ER-4111.

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LABS PERFORMING PIVOTAL STUDIES

A) Dept. of Drug Safety Research
Eisai Co, LTD
Japan

1) 3 month rat and dog toxicity
2) Segment I and III rat reproduction
3) Ames test

B)

1 year rat and dog toxicity

C)

1) Segment II reproduction in rats
2) Segment II reproduction in rabbits

D)

1) In vitro chromosomal aberration study
2) Mouse micronucleus test

PHARMACODYNAMICS:

These data will not be comprehensively reviewed at this time. Attached is a portion of my Original Summary of 1/24/91 for INDC which reviews much of the pharmacodynamics data for this drug. Also attached is the sponsor's NDA narrative summary and summary tables, which includes studies submitted subsequent to my original review; some results of interest from these studies are as follows (also see "Summary" section of the present review):

A) Mechanism of AchE inhibition

1) The inhibition by E-2020 (and also tacrine and physostigmine, but not DFP)in rat brain homogenate was dialyzable, indicating reversibility (see attached table).

2) Kinetic analysis in mouse brain homogenate indicated noncompetitive antagonism for both E-2020 and tacrine (see attached figures).

3) Kinetic analysis in electric eel led to a conclusion of a "mixed-type" inhibition (see attached figure) with binding of drug to both free and acetylated enzyme. (A similar conclusion was reached for tacrine).

B) Receptor binding profile

(Studied in rat brain; also opiate receptor binding in guinea pig brain).

Results shown in attached tables. No significant affinity was seen for any of the receptors studied except for a moderate affinity for sigma receptors in rat brain (IC 50 = 48 nM)

C) Inhibition of rat brain AchE in vitro by E2020 and several putative metabolites.

Results shown in attached table. Metabolites M-1 and M-3 had potencies similar to that of the parent compound; the others were 2 or more orders of magnitude less potent.

2. Effect of Membrane Dialysis on AChE inhibition

As shown in Table 3 (Appendices 5-8) the inhibitory action of 3, 10 and 30 nM E2020 disappeared after dialysis with AChE activities of 102, 98 and 97% control, respectively. Likewise, the inhibitory effects of PHY and THA were abolished by dialysis, indicating that all three compounds are reversible inhibitors of AChE. In contrast, the inhibitory effect of DFP 1, 3 and 10nM (45, 20 and 2% of control AChE activity respectively, before dialysis) was not affected by dialysis (40, 15 and 1% respectively, after dialysis), confirming the irreversible nature of the inhibitory action of this compound.

Table 3 Effect of Membrane Dialysis on Rat Brain AChE Inhibition Produced by E2020 and Reference Compounds.

Compound	Concentration (nM)	AChE Activity (% of Control)	
		Before Dialysis	After Dialysis
Control	—	100	100
E2020	3	69	102
	10	38	98
	30	19	97
THA	300	23	100
	1000	10	98
	3000	4	99
PHY	1	44	96
	3	33	101
	10	20	102
DFP	1	45	40
	3	20	15
	10	2	1

AChE activity was assayed in duplicate.
Multiple observations were averaged and the percent of control activity was calculated from the mean (n=3).

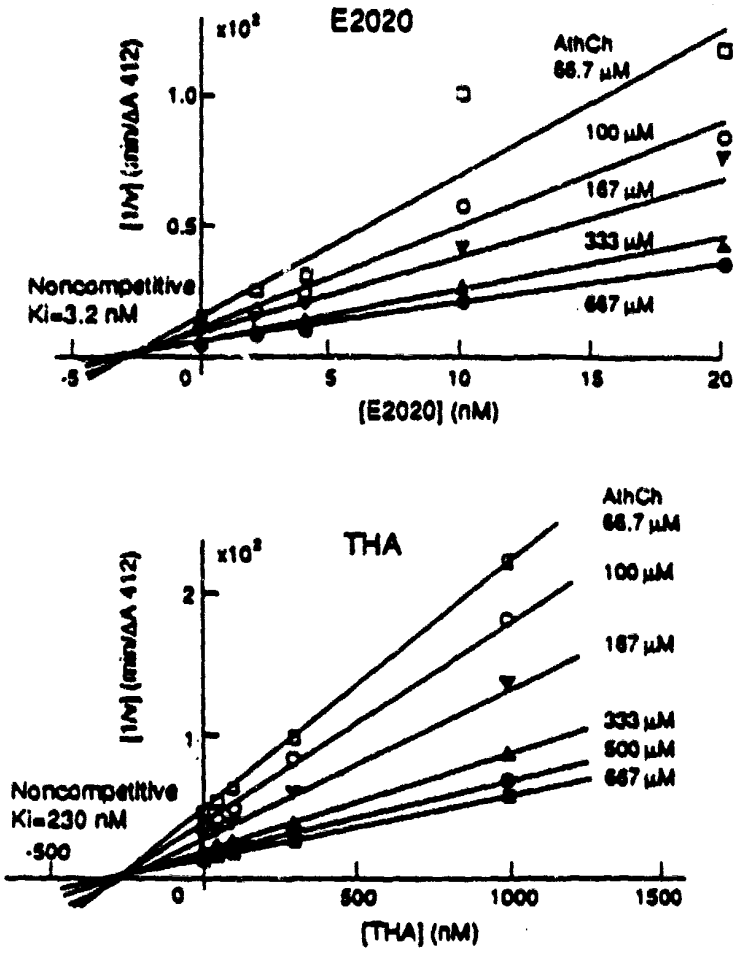

Figure 4 **Dixon Analysis of the Inhibition of Mouse Brain AChE by E2020 and THA.** Each point represents the mean from two observations. The K_i values for each drug are shown above the y axis.

Study W-880696 Final Report
Study W-19932632 Final Report
Study W-880707A Final Report

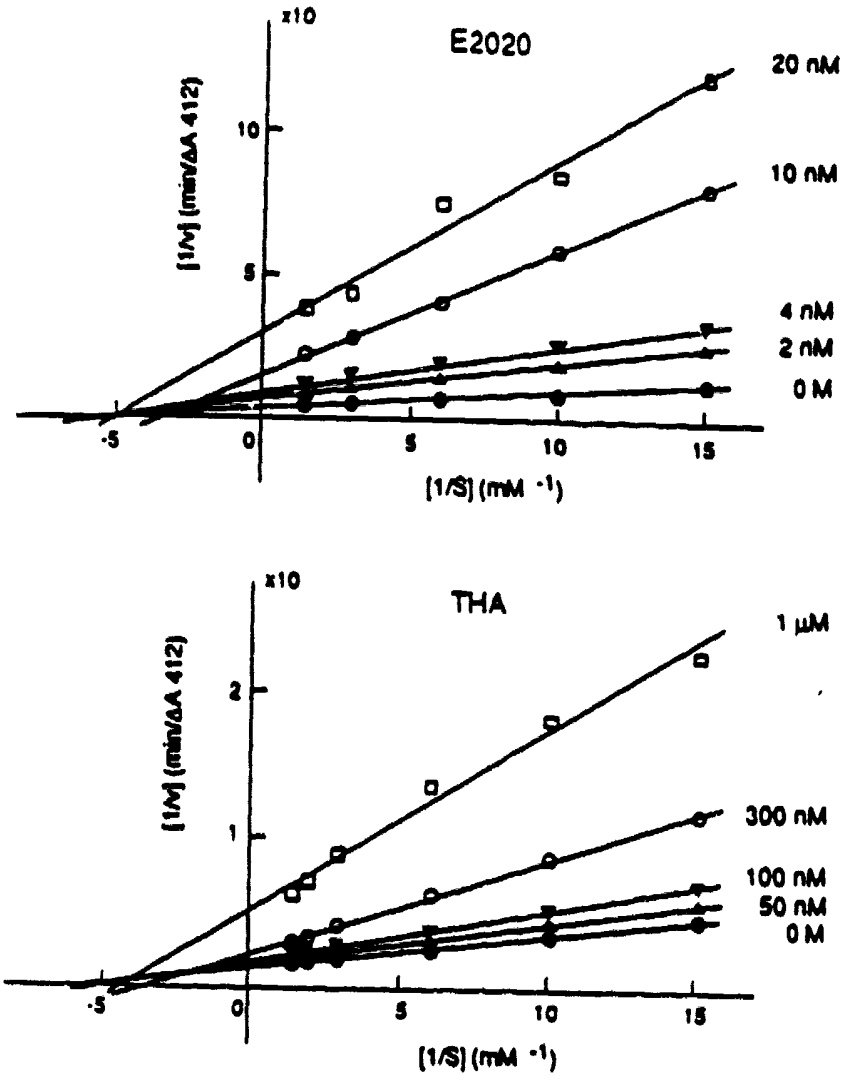

Figure 3 **Lineweaver-Burk Analysis of the Inhibition of Mouse Brain AChE by E2020 and THA.**

Each point represents the mean from two observations.

E2020: V_{max} 0.23 ΔA412/min K_m 1.1 x 10^{-4} M

THA : V_{max} 0.11 ΔA412/min K_m 2.9 x 10-4 M

4C

ELECTRIC EEL

RESULTS

The relationship between the concentrations of substrate and test compounds, and enzyme activities were analyzed using Lineweaver-Burk plots. As shown in Figs. 3 and 4, E2020 and THA showed inhibitory activity that fell into the category of "mixed type".

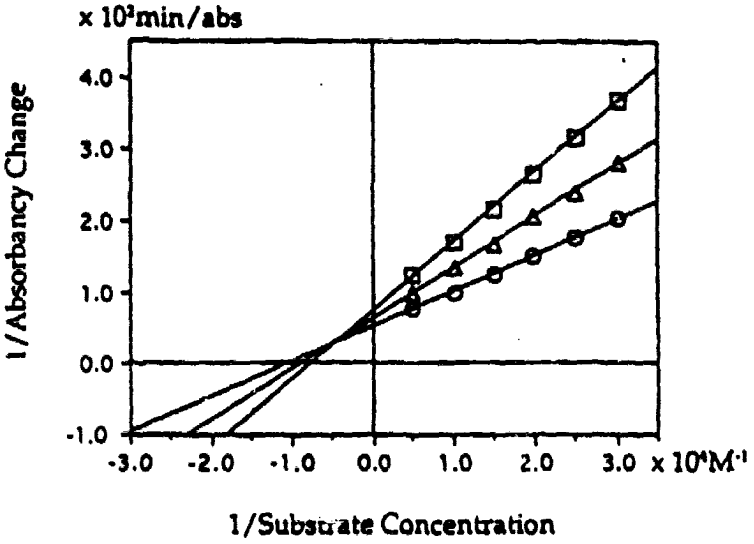

Fig. 3 Kinetics of acetylcholinesterase inhibition by E2020 using a Lineweaver-Burk plot

E2020 concentration: (○) zero M, (△) 2.00 x 10^{-9}M, (□)3.99 x 10^{-9}M

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RAT BRAIN RECEPTOR BINDING W-19950001

Table 1 Assay conditions for radioligand binding

Receptor	^{3}H-Ligand Name conc.	Tissue Brain area	Assay conditions Buffer, pH temp., time	Blank Compound Conc.	Ref
5-HT_1	[^{3}H]5-HT 1.9 nM	Cortex	A, pH 7.6, 37°C, 10 min	5-HT 10 µM	2
5-HT_2	[^{3}H]Ketanserin 0.2 nM	Cortex	B, pH 7.4, 37°C,15 min	Methysergide 1 µM	3
5-HT_3	[^{3}H]BRL 43694 0.5 nM	Cortex	B, pH 7.4, 37°C,30 min	Zacopride 10 µM	4
DA-D_1	[^{3}H]SCH23390 0.3 nM	Striatum	C, pH 7.4, RT, 60 min	SCH23390 10 µM	5
DA-D_2	[^{3}H]Spiperone 0.7 nM	Striatum	C, pH 7.4, RT, 60 min	Spiperone 10 µM	6
α_1-Adr	[^{3}H]Prazosin 0.2 nM	Cortex	B, pH 7.4, RT, 60 min	Phentolamine 10 µM	7
α_2-Adr	[^{3}H]Clonidine 4.5 nM	Cortex	B, pH 7.4, RT, 60 min	Phentolamine 10 µM	8
β-Adr	[^{3}H]Dihydroalprenolol 1.2 nM	Cortex	B, pH 8.0, RT, 20 min	(-)Alprenolol 1 µM	9
Histamine-H_1	[^{3}H]Mepyramine 2 nM	Cortex	D, pH 7.5, RT, 30 min	Promethazine 2 µM	10
Muscarinic acetylcholine	[^{3}H]QNB 0.7 nM	Striatum	C, pH 7.4, RT, 30 min	Atropine 1 µM	11
$GABA_A$	[^{3}H]Muscimol 5 nM	Corte	E, pH 7.1, 0°C, 60 min	GABA 100 nM	12
Benzodiazepine	[^{3}H]Flunitrazepam 0.8 nM	Cortex	B, pH 7.4, RT, 20 min	Diazepam 10 µM	13
Sigma	[^{3}H]DTG 2.4 nM	Cortex	B, pH 7.4, RT, 90 min	Haloperidol 10 µM	14

Buffers A: 50 mM Tris-HCl containing 10 µM pargyline, 5.7 mM ascorbate, 4 mM $CaCl_2$. B: 50 mM Tris-HCl. C: 50 mM Krebs-Tris. D: 50 mM phosphate containing 0.1% ascorbic acid. E: 50 mM Tris-citrate
Abbreviations: RT, room temperature; DTG, 1,3-di-O-tolylguanidine; QNB, quinuclidinyl bensylate; 5HT, 5-hydroxytryptamine receptor; DA, dopamine receptor; Adr, adrenoceptor; GABA, γ-aminobutyric acid

RAT BRAIN RECEPTOR BINDING

RESULTS

The affinities of E2020 and THA for the receptors examined are shown in Table 4. E2020 and THA showed practically no affinity for α_1-adrenoceptor, α_2-adrenoceptor, β-adrenoceptor, 5-HT_1, 5-HT_2, 5-HT_3, dopamine 1, dopamine 2, histamine 1, muscarinic cholinergic, $GABA_A$, and benzodiazepine receptors. E2020 showed, however, a moderate affinity (IC_{50} : 48.1 nM) for the sigma receptor while THA had no affinity.

Table 4 IC_{50} values of E2020 and THA for various receptors

Receptor	IC_{50} (nM)	
	E2020	THA
5-HT_1	>10,000	>10,000
5-HT_2	>10,000	>10,000
5-HT_3	>10,000	>10,000
DA-D_1	>10,000	>10,000
DA-D_2	>10,000	>10,000
α_1-Adr	3750	7300
α_2-Adr	>10,000	>10,000
β-Adr	>10,000	>10,000
Histamine-H_1	>10,000	>10,000
Muscarinic acetylcholine	1530	3670
$GABA_A$	>10,000	>10,000
Benzodiazepine	>10,000	>10,000
Sigma	48.1	>10,000

Abbreviations: 5-HT, 5-hydroxytryptamine receptor; DA, dopamine receptor; Adr, adrenoceptor; GABA, γ-aminobutyric acid

and centrifuged at 1,000 xg for 10 minutes. The supernatant obtained was recentrifuged at 20,000 xg for 20 minutes. The pellet obtained was washed and then resuspended in 10 volumes of 50mM Tris-HCl (pH7.6). The suspension was incubated for 10 minutes at 37°C in order to degrade endogenous opioid peptides, and then recentrifuged at 20,000 xg for 20 minutes. The pellet obtained (P2 fraction) was washed and resuspended in the same buffer. This receptor source was stored at -80°C prior to use.

3. Receptor binding assay

The receptor source was thawed and resuspended using a physcotron. Each assay was carried out in duplicate by incubating aliquots of the receptor source with a tritiated ligand, with or without test compounds, and with or without 10 μM levallorphan, in assay buffer (50mM Tris-HCl, pH7.6) in a final volume of 0.5 ml. Incubation was terminated by rapid filtration of the mixture through Whatman GF/B glass filters presoaked in 0.3% polyethyleneimine, using a

). Filters were rinsed with ice-cold 50mM Tris-HCl (pH 7.6), placed in vial, and 5 ml of ACS II scintillant

was added. The retained radioactivity was counted with a liquid scintillation counter

. Specific binding was calculated by subtracting non-specific binding measured in the presence of 10μM levallorphan. IC_{50} values were calculated by probit analys.

4. Radioligands

Radioligands used are shown in Table 2.

Table 2 Tritiated-labeled ligands

subtype	ligand	Lot.No.	specific activity (Ci/mmol)
μ	DAMGO (NET-902)	3186-043	55.5
κ	U-69,593 (NET-952)	3117-171	47.4
δ	DPDPE, 4-p-Cl-Phe (NET-923)	3164-047	48.6

is Tyr-D-Ala-Gly-N-Methyl-Phe-Gly-ol, U-69,593 is (5α,7α,8β)-

(+)-*N*--methyl-*N*--[7-(1-pyrrolidinyl)-1-oxaspiro [4,5]dec-8-yl]-benzenacetamide and DPDPE, 4-p-Cl-Phe is enkephalin, (2,5-D-penicillamine, 4-Cl-phenylalanine).

5. Unlabeled compounds

Levallorphan (10μM) was used for the calculation of non-specific binding(NSP).

OPIATE RECEPTOR BINDING, CONT'D

Results

The affinities of E2020 and THA for the receptors examined are shown in Table 3. E2020 and THA showed no affinity for opioid receptors (μ, κ and δ). Morphine showed selective affinity for the μ-subtype opioid receptor. Levallorphan showed nonselective and high affinity for opioid receptors. Individual and calculated binding data for all compounds and ligands are presented in Appendix 1.

Table 3 Opioid receptor IC_{50} values for E2020, THA, morphine, and levallorphan

	μ	κ	δ
E2020	380000±370000	1750000±2440000	112000±84000
THA	46200±5200	102000±54000	>2000000
morphine	24.4±10.9	764±245	440±479
levallorphan	5.38±1.92	3.23±1.23	6.66±3.86

Data are presented as mean (nM) of three experiments ± S.D.

Conclusion

The results of this study show that E2020 has no affinity for opioid receptor subtypes μ, κ, or δ.

References

1. Chang K.J., Cooper B.R., Hazum E. and Cuatrecasas P.: Multiple opiate receptors: Different regional distribution in the brain and differential binding of opiates and opioid peptides. Mol. Pharmcol. *16:* 91-104 (1979)
2. Lahti R.A., Mickelson M.M., McCall J.M. and VonVolgtlander P.F.: [^{3}H]U-69593; a highly selective ligand for the opioid κ receptor. Eur. J. Pharmacol. *109:* 281-284 (1985)
3. Toth G., Kramer T.H., Knapp R., Lui G., Davis P., Burks T.F., Yamamura H.I., and Hruby V.: [D-Pen^2,D-Pen^5]enkephalin analogues with increased affinity and selectivity for δ opioid receptors. J. Med. Chem. *33:* 249-253 (1990)

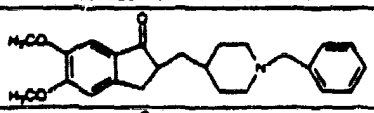
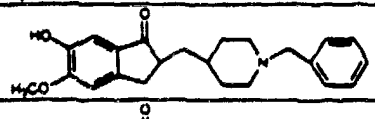
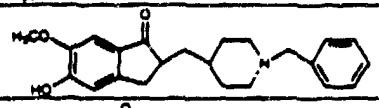
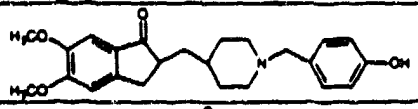
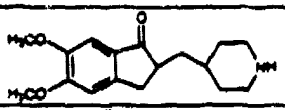
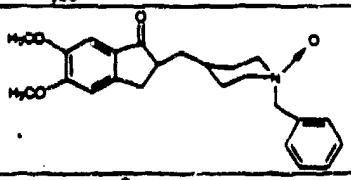
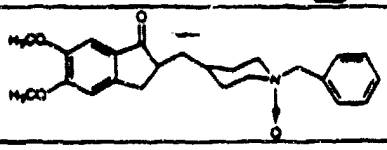
METABOLITES – AChE inhibition

Results

The inhibitory effects of E2020 and six of its putative metabolites on AChE *in vitro* are shown in Table 1. The IC_{50} values for AChE inhibition of E2020, M1, M2, M3, M4, M5, and M6 were 4.9 ± 0.1, 6.0 ± 0.3, 1131 ± 27, 2.4 ± 0.2, 9246 ± 845, 390 ± 4, and 314 ± 7 nM, respectively.

Two metabolites, M1 and M3, among the tested metabolites showed almost the same potency as E2020 in inhibition of AChE *in vitro*. The other metabolites, M2, M4, M5, and M6 were 64 to 1887 times less potent than E2020.

Table 1 Inhibitory effects of E2020 and its putative metabolites on rat brain AChE *in vitro*.

Compound	Chemical Structure	IC_{50} (nM)
E2020		4.9 ± 0.1
M-1		6.0 ± 0.3
M-2		1131 ± 27
M-3		2.4 ± 0.2
M-4		9246 ± 845
M-5		390 ± 4
M-6		314 ± 7

Values represent the mean ± SE from 4 dose - response curves for each test compound.

SPONSOR'S SUMMARY (PD)

Non Clinical Pharmacology and Toxicology

Overall Summary of Pharmacology

Primary Activity: Neuropharmacology Studies

Studies have been conducted *ex vivo*, *in vivo*, and *in vitro* using intact mice and rats, or systems derived from mice, rats or the electric eel, to determine the primary effects of E2020 on the peripheral and central cholinergic systems.

Effects on Cholinesterase Activity

In Vitro Studies

Unlike two recognized cholinesterase inhibitors, physostigmine (PHY) and tetrahydroaminoacridine (THA), E2020 was a much more selective inhibitor of acetylcholinesterase (AChE) than butyrylcholinesterase (BuChE; pseudocholinesterase) *in vitro* [Study Reference MP-1]. Both E2020 and THA were noncompetitive inhibitors in rat brain homogenates and all three acted reversibly.

E2020 and THA were shown [Study Reference MP-2] to be potent *in vitro* inhibitors of electric eel AChE. A steady-state analysis indicated that E2020 binds to both the free and acetylated enzyme, and may inhibit the deacetylation step during the AChE catalytic cycle.

Of putative E2020 metabolites M1, M2, M3, M4, M5 and M6, only M1 and M3 exhibited similar potency to E2020 in the inhibition of AChE from rat brain *in vitro* [Study Reference MP-3].

E2020 is a chiral molecule. When examined individually [Study Reference MP-4], the R- and S- enantiomers were both found to inhibit mouse brain AChE *in vitro*, with R-E2020 being more potent than S-E2020.

Ex Vivo Studies

A series of studies was conducted to determine the effect of E2020, PHY and THA on ChE activity in the brain and in other tissues obtained from orally treated animals.

Compared *ex vivo* [Study Reference MP-5] in tissues obtained from the rat, E2020 produced a greater degree of dose-dependent inhibition of brain AchE activity than either PHY or THA. At equal doses, E2020 had a longer duration of inhibitory action than PHY. While PHY and THA inhibited ChE in both brain and in almost all peripheral tissues, E2020 was far more selective, inhibiting ChE in brain and serum (reflecting the higher level of AChE in rat serum as compared to humans) significantly, but not in heart and small intestine, and only marginally in pectoral muscle.

When administered to aged rats, a model population appropriate for their ultimate use, E2020 and THA showed greater cholinesterase inhibitory activity than in young rats [Study Reference MP-6]. The difference was attributed to higher plasma and tissue concentrations, possibly the result of diminished activity of drug metabolizing enzymes in aged animals.

Both the R- and S- enantiomers of E2020 were shown to reduce AChE activity in rat brain *ex vivo* [Study Reference MP-7]. Residual AChE activity following administration of R-E2020 was slightly less than for the S- enantiomer.

Effects on Brain ACh Concentrations

E2020 and the known AChE inhibitors, PHY, THA and RA-6, caused a significant increase in the concentration of ACh in whole rat brain and E2020 increased local ACh concentrations in the cerebral cortex, hippocampus and striatum significantly [Study Reference MP-8]. E2020 also increased ACh in the extracellular space of the rat cerebral cortex significantly in a time- and dose-dependent manner and with greater potency than THA [Study Reference MP-9].

Effects on ACh Concentration in the Cerebral Cortex of Animals with Cerebral Cholinergic Dysfunction

Using three different models of cholinergic hypofunction, [Study Reference MP-10], E2020 was shown to increase concentrations of ACh in the brains of rats with abnormally low levels of ACh. A single oral dose of E2020 was more potent than THA in causing dose-dependent increases in cortical ACh in NBM-lesioned rats. The temporary depletion of brain ACh induced by the injection of scopolamine was blocked more strongly by prior administration of E2020 than THA. The decrease in hippocampal ACh caused by prior injection of the neurotoxin AF64A into both lateral ventricles of the rat brain was reversed significantly by oral doses of E2020, but not by THA.

Effects on the Central Cholinergic System

The ability of E2020 to stimulate the central cholinergic system was compared with that of THA, PHY and distigmine, using the rat yawning model [Study Reference MP-11]. E2020, THA and PHY elicited yawning dose-dependently. Since E2020-induced yawns were completely abolished by scopolamine, a centrally acting anticholinergic, but were not significantly affected by methylscopolamine, a peripheral anticholinergic agent, it was concluded that E2020 elicited yawning through a central cholinergic action and was more potent than THA or PHY in this regard.

Effects in Behavioral Models of Cholinergic Function

Several animal models related to learning and memory were used to determine the ability of E2020 to alter impaired behavior attributable to a deficiency in cortical ACh.

Oral E2020 was more effective than THA in reversing the effects of scopolamine-induced impairment of spatial memory in the rat, using performance in a radial maze as a model [Study Reference MP-12].

E2020 was more effective than THA in increasing response latency in a passive

avoidance task, a test of short-term memory [Study Reference MP-13]. NBM-lesioned rats, a model for Alzheimer's Disease, were trained to avoid the dark side of a two-compartment, light-dark chamber, using electric shock as a reinforcing stimulus. Lesioned animals pretreated with E2020 at doses from 0.125 to 1.0 mg/kg one hour prior to training showed a significant ($p<0.01$-0.05) increase in response latency when retested to assess memory retention 24 hours post-training. Animals treated with THA at doses from 0.25 to 1.0 mg/kg showed no significant increases in response latency. Thus, E2020 partially reversed impairment in retention of a passive avoidance task produced by lesions of the NBM.

Increased hyperlocomotion in a new or dark environment is a characteristic behavior attributed to decreased ACh concentrations in the brains of NBM-lesioned rats. When compared with several other cholinergic agents and dopamine antagonists [Study Reference MP-14], E2020 was more effective than THA in partially reversing hyperlocomotion by lesioned rats placed in a new environment.

Effects on Cholinergic Receptors

Since E2020 is expected to be used in chronic therapy, the effects of E2020 (3mg/kg or 20 mg/kg) on muscarinic receptors in the brain of rats were assessed after daily treatment for 13 consecutive weeks [Study Reference MP-15]. At 20 mg/kg/day, E2020 caused a small (14%), but signficant decrease in the density (Bmax) of muscarinic receptors. No effects on receptor affinity or number were detected following 13-weeks of treatment with 3 mg/kg/day E2020.

Studies Related to Possible Adverse Reactions

Neuropharmacological Studies

E2020, THA and PHY caused changes in the electroencephalograms (EEG) of conscious rabbits consistent with central arousal, considered to be a result of increased cholinergic stimulation from the inhibition of AChE [Study Reference GP-1].

The effects of doses of E2020 previously established as inhibitory of centrally mediated AChE activity were determined and compared with THA and PHY in five animal models [Study Reference GP-2]. (1) Increases in ACh caused by E2020 and THA had no effect on spontaneous motor activity in normal rats; (2) the level of ChE inhibition produced by E2020 and PHY did not reduce pentobarbital-induced sleep time in mice; (3) E2020 did not raise the threshold for tonic convulsions and death after application of alternating current to the corneas of mice; (4) E2020 did not block pentylenetetrazole-induced convulsions in mice; (5) E2020, at the highest dose tested (and considerably higher than those required for AChE inhibition), lengthened survival time marginally following KCN injection. Thus, E2020 has no marked activity as assessed in these models.

Using tritiated ligands, the receptor binding of E2020 in rat brain was examined *in vitro* to characterize the scope of its central activity [Study Reference GP-3]. The absence of significant binding to all but the sigma receptor suggests that the ability of E2020 to attenuate behavioral impairment induced by central cholinergic hypofunction in rats is mediated by inhibition of AChE rather than activation of receptors in the brain. Although E2020 had moderate affinity for the sigma receptor, no physiological or biochemical function has been conclusively associated with this receptor.

Neither E2020 nor THA showed binding affinity for the three opioid receptor subtypes, mu (μ), kappa (κ), or delta (δ), in guinea-pig brain [Study Reference GP-4]. Morphine showed selective affinity for the μ- subtype opioid receptor. Levallorphan showed nonselective and high affinity for all three opioid receptors.

E2020 was a much less potent inhibitor of monoamine (MA) uptake into rat brain synaptosomes *in vitro* than an inhibitor of AChE, suggesting that inhibition of MA uptake does not contribute significantly to the activity of E2020 *in vivo* [Study Reference GP-5].

The peripheral anticholinesterase effects of E2020 (e.g., skeletal muscle fasciculations, miosis, lacrimation, salivation and diarrhea) were similar to those of THA and PHY [Study Reference GP-6]. Miosis and fasciculation were observed consistently for all

three compounds.

Cardiovascular Studies

In anesthetized female mongrel dogs, E2020 produced no notable alterations in heart rate, cardiac contractility, arterial blood pressure or ECG after intravenous doses of up to 1 mg/kg [Study Reference GP-7]. These findings are consistent with studies in the rat, in which E2020 at oral doses of up to 3 mg/kg produced no statistically significant decreases in ChE activity in cardiac tissue [Study Reference MP-6].

E2020, THA and the platelet aggregation inhibitor, E5510, were tested *in vitro*, using human platelet-rich plasma and collagen as the inducer of platelet aggregation [Study Reference GP-8]. E2020 had no effect at concentrations achieved in patients (*ca.* 150 nM).

Gastrointestinal Studies

E2020 had no significant effect on intestinal transit in mice at doses of 1, 3 and 10 mg/kg [Study Reference GP-9]. In pylorus-ligated rats, E2020 did not stimulate gastric acid secretion, and actually decreased acid concentration, at doses as high as 10 mg/kg, a dose greater than that intended for clinical administration [Study Reference GP-10]. Pilot study results suggest that both E2020 and THA may produce dose-dependent increases in the frequency of gastric, but not duodenal contractions, in conscious rats [Study Reference GP-11].

Genitourinary Studies

In conscious rats, E2020 caused no significant effects on renal activity other than increases in urinary volume and Na^+ excretion at a dose of 10 mg/kg [Study Reference GP-12].

The relationship between the diuretic effects of E2020 and its activation of central cholinergic mechanisms was established in a second study [Study Reference GP-13].

E2020-induced diuresis and natriuresis during the first four hours after treatment were offset by a reduction in renal electrolyte excretion and urine volume during the ensuing 20 hours. Scopolamine (SC), a centrally acting anticholinergic agent, antagonized the early diuresis and natriuresis caused by a 10 mg/kg oral dose of E2020. Methylscopolamine (MSC), a predominantly peripheral anticholinergic agent, was a much weaker antagonist of the renal effects of E2020, confirming that these effects were due the inhibition of central AChE activity by E2020.

Endocrine Studies

E2020 at 1 and 3 mg/kg had no effect on either basal blood glucose concentrations or glucose tolerance; E2020 at 10 mg/kg, and THA at 10 and 30 mg/kg increased basal blood glucose concentration and reduced glucose tolerance [Study Reference GP-14].

Potential Interactions with Other Drugs

In normal rats, E2020 showed no effect on (1) the anticoagulant action of warfarin [Study Reference GP-15], (2) the diuretic action of furosemide [Study Reference GP-16], or (3) hypoglycemia produced by tolbutamide [Study Reference GP-17].

E2020: NONCLINICAL PHARMACOLOGY

Table of Studies

(Section 5 . B . 3 . 0)

I. Pharmacology Studies
a. Effects Related to the Therapeutic Indication (Primary Pharmacodynamics)

Study Reference	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observations	GLP Status; Laboratory
MP-1[1]	Inhibitory Effects of E2020 on Cholinesterase Activities *In Vitro* (Report No. W-880708A)	Volume 1.024 Page 1	Rat/ Wistar	Male/ Brain Homogenate	in vitro	3, 10, 30 nM	single incubation	E2020 is a selective, reversible, and non-competitive inhibitor of AChE. E2020 IC_{50}=5.70 nM; Ki=3.2 nM	Non GLP; Eisai Tsukuba Research Labs, Japan
MP-2[2]	Kinetics of Electric Eel Acetylcholinesterase Inhibition by E2020 (Report No. W-880996J)	Volume 1.024 Page 37	Electric Eel	Purified AChE	in vitro	0, 2, 3.99 nM	single incubation	E2020 inhibited AChE and demonstrated a "mixed-type" inhibition. E2020 Ki=4.45 nM; E2020 Ki'=9.52 nM	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-3[2]	Inhibitory Effects of E2020 and Six of its Putative Metabolites on Acetylcholinesterase From Rat Brain *In Vitro* (Report No. W-19952682)	Volume 1.024 Page 56	Rat/ Wistar	Male/ Brain Homogenate	in vitro	1, 3, 10 nM	single incubation	IC_{50} values of metabolites M-1 and M-3 are comparable to E2020 in inhibiting rat brain AChE. E2020 IC_{50}=4.9 nM	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-4[2]	Inhibitory Effects of R-, S- and R, S-E2020 on Mouse Brain Cholinesterase Activity *In Vitro* (Report No. W-880702A)	Volume 1.024 Page 71	Mouse/ ddy	Male/ Brain Homogenate	in vitro	3, 10 nM	single incubation	IC_{50} values for S-, R-, and R-S E2020 are 7.7, 4.8, and 5.9 nM respectively.	Non-GLP; Eisai Tsukuba Research Labs, Japan

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I. Pharmacology Studies
a. Effects Related to the Therapeutic Indication (Primary Pharmacodynamics)

Study Reference	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observations	GLP Status; Laboratory
MP-5[1]	Inhibitory Effects of Orally-Administered E2020 on Cholinesterase Activity in Rats (Report No. W-890708)	Volume 1.024 Page 80	Rat/ Wistar	6 groups of 6 males 3 groups of 5-6 males 3 groups of 6 males	p.o. p.o. p.o.	0, 0, 1, 3, 10, 30 mg/kg 0, 5, 10 mg/kg 0, 3, 10 mg/kg	single dose	E2020 significantly inhibited AChE activity at all doses with a duration up to 8 hrs at 10 mg/kg. Among the tissues examined, E2020 only inhibited ChE of the brain & serum (3, 10 mg/kg) and pectoral muscle (10 mg/kg).	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-6[1]	Effects of E2020 on Cholinesterase Activity in Young and Aged Rats (Report No. W-910698)	Volume 1.024 Page 113	Rat/ Fisher 344	6 groups of 5 males (young vs. aged)	p.o.	0, 3, 10 mg/kg	single dose	E2020 inhibited AChE in a dose-related manner. Inhibition was more pronounced in aged than young animals and E2020 was more selective for cerebral tissue than smooth muscle than THA.	Non-GLP; Eisai CNS Research Unit, Japan
MP-7[1]	Inhibitory Effects of Orally-Administered R- and S-E2020 on Brain Cholinesterase Activity in Rats Measured Ex Vivo (Report No. W-920702S)	Volume 1.024 Page 144	Rat/ Wistar	3 groups of 6 males	p.o.	3, 10 mg/kg	single dose	Both R- and S-E2020 isomers are potent inhibitors of brain AChE.	Non-GLP; Eisai Tsukuba Research Labs, Japan

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I. Pharmacology Studies
a. Effects Related to the Therapeutic Indication (Primary Pharmacodynamics)

Study Reference	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observations	GLP Status; Laboratory
MP-8[1]	Effects of E2020 on Acetylcholine Concentration in Rat Brain (Report No. W-880755)	Volume 1.024 Page 152	Rat/ Wistar	3 groups of 6 males 5 groups of 6 males	p.o. p.o.	0, 5, 20 mg/kg 0, 1.25, 2.5, 5, 10 mg/kg	single dose	E2020 increased ACh concentrations in the whole brain and discrete regions of the brain (cortex, hippocampus, and striatum).	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-9[1]	Effect of E2020 on the Level of Extracellular Acetylcholine in the Cerebral Cortex of Rats (Report No. W-890908)	Volume 1.024 Page 168	Rat/ Wistar	2 groups of 4-5 males	i.p.	1, 3 mg/kg	single dose	At all doses, E2020 increased ACh concentrations in the extracellular space of the cerebral cortex.	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-10[1]	Effects of E2020 on Brain Acetylcholine Levels in Three Rat Models of Cholinergic Hypofunction (Report No. W-891133)	Volume 1.024 Page 195	Rat/ Wistar	5 groups of 6-7 males 4 groups of 6 males 4 groups of 6 males	p.o.	0, 1.25, 2.5, 5, 10 mg/kg 0, 2.5, 5, 10, mg/kg 0, 1.25, 2.5, 5 mg/kg	single dose	E2020 antagonized the reduction of ACh concentration in a dose-dependent manner in three rat models.	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-11[2]	Effects of E2020 in Eliciting Yawning in Rats (Report No. W-890963J)	Volume 1.024 Page 212	Rat/ Wistar	6 groups of 10 males	p.o.	0, 0.5, 1, 2, 4, 8 mg/kg	single dose	E2020 induced yawning dose-dependently at ≥ 1 mg/kg.	Non-GLP; Eisai CNS Research Unit, Japan

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I. Pharmacology Studies
a. Effects Related to the Therapeutic Indication (Primary Pharmacodynamics)

Study Reference/ Publication	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observations	GLP Status; Laboratory
MP-12[1]	Effect of E2020 on Scopolamine-Induced Impairment on Performance of the Spatial Memory Task in Rats (Report No. W-890716)	Volume 1.024 Page 230	Rat/ Wistar	3 groups of 10 males	p.o.	0, 0.25, 0.5 mg/kg	single dose	Pre-treatment with E2020 improved radial arm performance in rats with scopolamine-induced impairment.	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-13[1]	Effects of E2020 on the Impairment of Passive Avoidance in Rats After Destruction of the Nucleus Basalis Magnocellularis (Report No. W-890979)	Volume 1.024 Page 249	Rat/ Wistar	4 groups of 16-17 males	p.o.	0.125, 0.25, 0.5, 1.0 mg/kg	single dose	E2020 at 0.125, 0.25, and 1.0 mg/kg improved performance (increased latency) in animals with NBM lesions.	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-14[1]	Inhibitory Effects of E2020 on Hypolocomotion in Rats After Destruction of the Nucleus Basalis Magnocellularis (Report No. W-890655)	Volume 1.024 Page 264	Rat/ Wistar	2 groups of 4-6 males	s.c.	0, 2 mg/kg	single dose	E2020 decreased NBM-lesion induced hypolocomotion.	Non-GLP; Eisai Tsukuba Research Labs, Japan
MP-15[2]	Effect of Chronic Administration of E2020 on Muscarinic Cholinergic Receptors in the Rat Brain (Report No. W-890633J)	Volume 1.024 Page 300	Rat/ Sprague-Dawley	3 groups of 10 males	p.o.	0, 3, 20 mg/kg	single dose daily x 13 weeks	Chronic treatment with E2020 at 20 mg/kg caused down-regulation of muscarinic ACh receptors; there was no change in B_{max} or K_d following E2020 at 3 mg/kg.	Non-GLP; Eisai CNS Research Unit, Japan

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I. Pharmacology Studies

b. Effects Related to Possible Adverse Reactions

Study Reference	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observation	GLP Status; Laboratory
GP-1[1]	A Pilot Study of the Effects of E2020 on Electroencephalograms (EEG) in Rabbits (Report No. W-890975)	Volume 1.025 Page 1	Rabbit/ NIBS	5 males	i.v.	0, 0.125, 0.25, 0.5, 1 mg/kg	single incremental doses	Dose-related EEG arousal observed at 0.25 to 0.5 mg/kg E2020, with no effect at 0.125 mg/kg.	Non-GLP; Eisai CNS Research Unit, Tsukuba, Japan
GP-2[1]	General Pharmacological Study of the Effects of E2020 on the Central Nervous System of Mice and Rats (Report No. W-890475)	Volume 1.025 Page 9	Rat/ Wistar Mouse/ ddy	4 groups of 5 males 4 or 5 groups of 8-10 males	p.o.	0, 0.3, 1, 3, 10, 30 mg/kg	single dose	E2020 had no effect on spontaneous motor activity in normal rats, pentobarbital-induced sleep, the threshold for tolerance in electroshock-induced convulsions, and PTZ-induced convulsion at 10 mg/kg. E2020 increased the survival time in mice administered KCN.	Non-GLP; Eisai CNS Research Unit, Tsukuba, Japan
GP-3[#]	Receptor Binding Profile of E2020 in Rat Brain (Report No. W-19950001)	Volume 1.025 Page 29	Rat/ Sprague-Dawley	male Brain Homogenate	in vitro	1, 10, 100 μM	single incubation	E2020 showed no affinity for the receptors studied except moderate affinity for the sigma receptor (IC_{50} = 48.1 nM).	Non-GLP; Eisai CNS Research Unit, Tsukuba, Japan
GP-4[#]	Binding Affinity of E2020 for Opioid Receptor in Guinea Pig Brain (Report No. W-19952727)	Volume 1.025 Page 52	Guinea Pig/ Hartley	male Brain Homogenate	in vitro	2, 20, 200 μM	single incubation	E2020 had no affinity for opioid receptor subtypes (μ, K, δ).	Non-GLP; Eisai CNS Research Unit, Tsukuba, Japan

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I. Pharmacology Studies
b. Effects Related to Possible Adverse Reactions

Study Reference	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observations	GLP Status; Laboratory
GP-5[n]	Effects of E2020, a Novel Acetylcholinesterase Inhibitor, on Monoamine (MA) Uptake into Rat Brain Synaptosomes (Report No. W-19952724)	Volume 1.025 Page 65	Rat/ Wistar	male Brain Homogenate	in vitro	0, 0.1, 0.3, 1, 3, 10, 30, 100 μM	single incubation	E2020 inhibited [^{3}H]NE uptake with IC_{50} = 2.62 μM, [^{3}H]5-HT uptake with IC_{50} = 4.05 μM, and [^{3}H]DA uptake with IC_{50} = 4.19 μM.	Non-GLP; Eisai CNS Research Unit, Tsukuba, Japan
GP-6[n]	Development of Peripheral Symptoms after Oral Administration of E2020 to Rats (Report No. W-880628)	Volume 1.025 Page 88	Rat/ Wistar	5 groups of 4 males	p.o.	1.25, 2.5, 5, 10 mg/kg	single dose	Miosis and fasciculation were observed and both effects were dose-dependent; E2020 at 1.25 mg/kg produced no fasciculation.	Non-GLP; Eisai CNS Research Unit, Tsukuba, Japan
GP-7[1]	A Pilot Study of the Effects of E2020 on Cardiohemodynamics and ECG in Anesthetized Dogs (Report No. W-870721)	Volume 1.025 Page 112	Dog/ Mongrels	3 females	i.v.	0.1, 0.3, 1 mg/kg	single incremental doses	E2020 had no effects on arterial pressure, heart rate, cardiac contractility, and ECG in this dose range.	Non-GLP; Eisai Cardiovascular Unit, Tsukuba, Japan
GP-8[1]	Effects of E2020 on Platelet Aggregation in Human Platelet-Rich Plasma *In Vitro* (Report No. W-890933)	Volume 1.025 Page 122	Man	Human Platelets	in vitro	100, 200, 500 μM	single incubation	E2020 only showed inhibition of platelet aggregation at 500 μM.	Non-GLP; Eisai Thrombosis of Vascular Research Unit, Tsukuba, Japan

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I. Pharmacology Studies
b. Effects Related to Possible Adverse Reactions

Study Reference	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observations	GLP Status; Laboratory
GP-9[#]	Effects of E2020 on Intestinal Transit in Mice (Study No. G94023; Report No.W19960345)	Volume 1.025 Page 133	Mice/ ICR	4 groups of 8 males	p.o.	0, 1, 3, 10 mg/kg	single dose	E2020 had no significant effect on intestinal transit rate at any dose	
GP-10[!]	Effects of E2020 on Gastric Acid Secretion in Pylorus-Ligated Rats (Report No. W-870877)	Volume 1.025 Page 148	Rat/ Sprague-Dawley	4 groups of 6 males	ID	0, 1, 3, 10 mg/kg	single dose	E2020 had no effect on total acid secretion, but reduced gastric acid concentration at 10 mg/kg.	
GP-11[!]	A Pilot Study of the Effects of E2020 on Gastrointestinal Motility in Conscious Rats (Report No. W-870896)	Volume 1.025 Page 159	Rat/ Sprague-Dawley	3 groups of 5 males	ID	1, 3, 10 mg/kg	single incremental doses	E2020 increased gastric motility at all doses. The effects on duodenal motility was variable.	
GP-12[!]	Effects on E2020 on Urinary Water and Electrolyte Excretion in Conscious Rats (Report No. W-870825)	Volume 1.025 Page 169	Rat/ Sprague Dawley	4 groups of 6 males	p.o.	0, 1, 3, 10 mg/kg	single dose	At 10 mg/kg, E2020 exerts diuretic and natriuretic effects.	

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I. Pharmacology Studies
b. Effects Related to Possible Adverse Reactions

Study Reference	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observations	GLP Status; Laboratory
GP-13[1]	Effects of Scopolamine (SCP) and Methylscopolamine (MSCP) on the Diuretic Action of E2020 in Rats (Report No. W-880630)	Volume 1.025 Page 189	Rat/ Sprague Dawley	2 groups of 6 males	p.o.	0, 10 mg/kg	single dose	E2020 causes biphasic changes in salt excretion. The natriuretic effect was inhibited by SCP but to a lesser degree with MSCP.	Non-GLP; Eisai CNS and Cardiovascular Research Unit, Japan
GP-14[1]	Effects of E2020 on the Fasting Blood Glucose Level and Glucose Tolerance in Rats (Report No. W-870829)	Volume 1.025 Page 207	Rat/ Sprague-Dawley	4 groups of 4-5 males	p.o.	0, 1, 3, 10 mg/kg	single dose	E2020 increased basal glucose levels and reduced glucose tolerance at a high dose of 10 mg/kg.	Non-GLP; Eisai Thrombosis and Vascular Research Unit, Japan

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I. Pharmacology Studies
c. Interaction With Other Drugs

Study Reference	Title of Study:	Full Report Location	Species/ Strain	Group/ Number/ Gender	Route of Admin.	E2020 Doses	Duration	Observations	GLP Status; Laboratory
GP-15[#]	Effect of E2020 on the Anti-Coagulant Action of Warfarin in Male Rats (Study No. G94012; Report No. W-19952284)	Volume 1.025 Page 227	Rat/ Sprague Dawley	2 groups of 6 males	p.o.	0, 3 mg/kg	once daily x 2 days	E2020 had no effect on the anticoagulation action of warfarin.	GLP; Eisai Dept. of Preclinical Pharmacology, Tsukuba, Japan
GP-16[#]	Effect of E2020 on the Diuretic Action of Furosemide in Fasted Male Rats (Study No. G94024; Report No. W-19952285)	Volume 1.025 Page 246	Rat/ Sprague Dawley	4 groups of 7 males	p.o.	0, 3 mg/kg	single dose	E2020 had no effect on the diuretic action of furosemide.	GLP; Eisai Dept. of Preclinical Pharmacology, Tsukuba, Japan
GP-17[#]	Effect of E2020 on the Hypoglycemia Produced By Tolbutamide in Fasted Male Rats (Study No. G94010; Report No. W-19952283)	Volume 1.025 Page 265	Rat/ Sprague Dawley	4 groups of 6 males	p.o.	0, 3 mg/kg	single dose	E2020 had no effect on hypoglycemia caused by tolbutamide.	GLP; Eisai Dept. of Preclinical Pharmacology, Tsukuba, Japan

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END OF SPONSOR'S SUMMARY (PD)

FROM ORIGINAL PHARM SUMMARY OF IND

(1/24/91)

SUMMARY:

(E 20 20 abbreviated as E, physostigmine abbreviated as P).

A) Pharmacodynamics:

IC 50 values for inhibition of rat brain acetylcholinesterase (AchE) and rat plasma butyrylcholinesterase (BuChE) were as follows:

Table 1: Inhibitory Effects of E2020 and Reference Compounds on Rat Brain AChE and Rat Plasma BuChE *in vitro*.

Compound	IC_{50}(nM)		Ratio of IC_{50}s'
	AChE Activity	BuChE Activity	(BuChE/AChE)
E2020	5.70 ± 0.20	7138.0 ± 133	1252.0
PHY	0.68 ± 0.02	8.1 ± 0.3	11.9
THA	80.60 ± 2.50	73.0 ± 0.9	0.9

Values represent the mean ± S.E. from 4 dose-response curves for each test drug.

E was about 10x less potent than P ("PHY") and about 10x more potent than tacrine ("THA"). E had the greatest specificity of the 3 compounds for AchE (vs BuChE). It was stated that "more extensive kinetic studies" indicated that E is a reversible noncompetitive inhibitor of AchE, but no specifics were given.

The following results were obtained in an ex vivo study of AchE inhibition in rat brain (drugs given p.o. 1 hour prior to sacrifice):

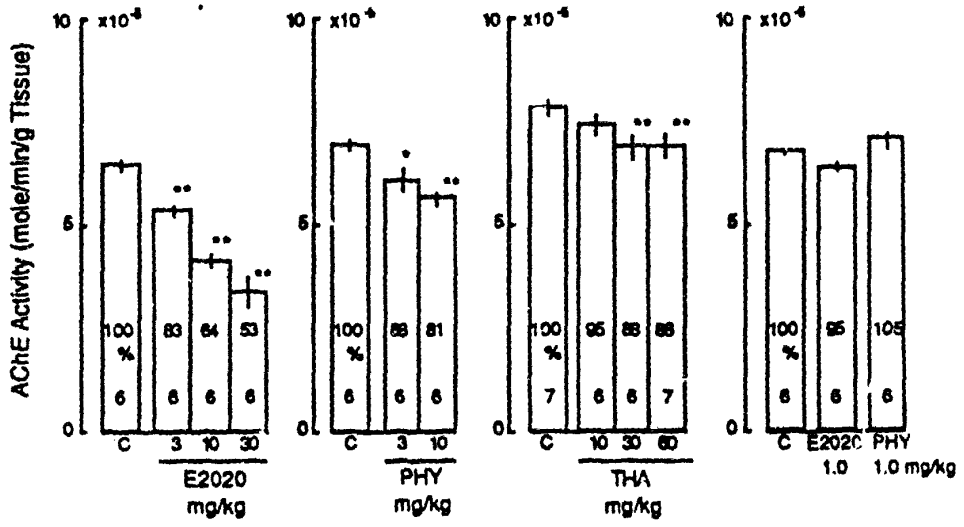

Figure 1: Effects of Oral Administration of E2020, Physostigmine (PHY) and Tetrahydroaminoacridine (THA) on Rat Brain AChE Activity *ex vivo*.

Each column denotes the mean ±S.E., *, **: $p<0.05$, $p<0.01$ vs. control. The top numbers in each column represent the percent inhibition relative to control. The lower numbers represent the number of animals used per group.

4 cc

E and P caused inhibition at 3 but not 1 mg/kg p.o. E appeared to be slightly more potent and/or effective than P and significantly more potent than THA, although this conclusion is tempered by the limited D-R data obtained and the single time point used. Nothing was stated regarding the toxic signs produced by each drug at these doses; this would have been useful since an important goal in this field is to develop drugs with therapeutic indeces greater than that of P.

E was shown to elevate rat brain acetylcholine (Ach) at a dose of 5 mg/kg p.o.; it was not stated if lower doses were used:

Table 2: Effects of E2020 on ACh Concentrations of the Cerebral Cortex, Hippocampus and Striatum of Wistar Rats.

Treatment	Dose mg/kg	n	ACh Concentration (nmole/g)		
			Cortex	Hippocampus	Striatum
Saline	-	6	15.8 ± 0.79	23.5 ± 0.74	67.7 ± 3.52
E2020	5	6	20.4 ± 0.69*	25.6 ± 0.78	87.8 ± 3.99
E2020	20	6	21.8 ± 1.44**	28.7 ± 1.14**	99.3 ± 8.95**

Figures are mean ± S.E.
*,**: $p<0.05$, 0.01 vs. respective control (Dunnett's t-test).

In another study, E at 1 or 3 mg/kg i.p. increased Ach release in rat cerebral cortex in vivo; this was statistically significant for up to 60 minutes:

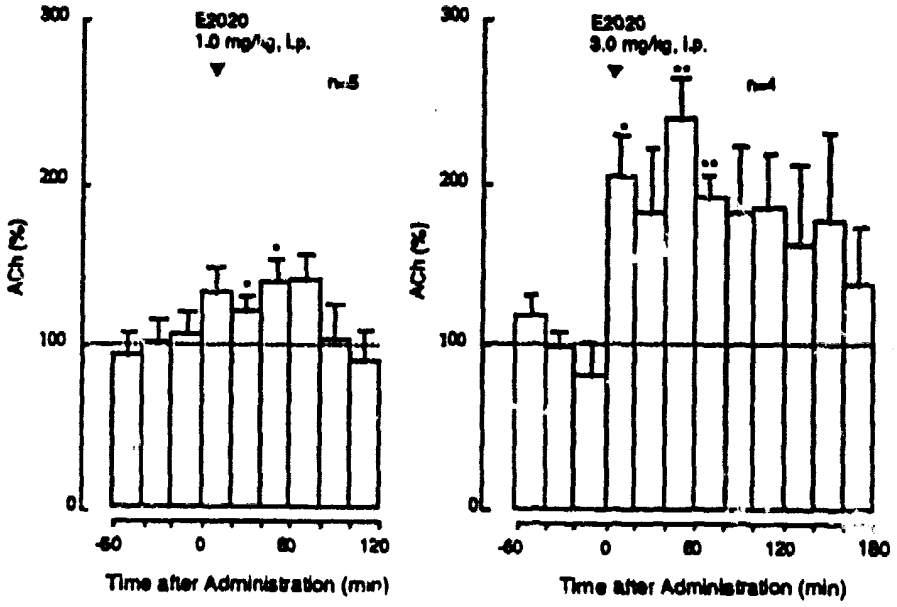

Figure 3: Effects of E2020 on Extracellular ACh Levels in the Cerebral Cortex of Rats.

Results are expressed as the mean % change from the average baseline value ± S.E. *,**: $p<0.05$, 0.01 vs. baseline release (paired t-test, n=4-5).

(Note that this does not necessarily show an increase in Ach release, since levels could be increased due to AchE inhibition. Also note that increases in Ach levels or release do not necessarily imply an increase in cholinergic neurotransmission).

Other studies showed that E, at doses as low as 1.25 - 5 mg/kg p.o., increased brain Ach level in rats whose brain Ach had been decreased by chemical lesions or scopolamine administration

E was active in 2 behavioral models of cholinergic hypofunction in rats: (1) at 0.25 - 0.50 mg/kg p.o. it partly antagonized scopolamine-induced increases in running time and error number in a radial maze task (THA was about 4x less potent than E) and (2) at 0.125 - 1 mg/kg p.o. (but not D-R) it partly antagonized NBM lesion-induced disruption of retention of a passive avoidance response. (THA inactive up to 1 mg/kg p.o.) (Note that in the latter study, E was given prior to training; thus an effect on retention per se cannot be concluded). In another study E at 2 mg/kg s.c. in rats partly antagonized scopolamine-induced increases in locomotor activity; the effect of E alone was not studied.

E
P was inactive in the following studies of CNS activity (maximum dose in mg/kg in parentheses): spontaneous motility in rats (3 p.o.), pentobarbital sleep time in mice (10 p.o.), electroshock-induced convulsions in mice (10 p.o.), and pentylentetrazol-induced convulsions in mice (10 p.o.). E at 10 but not 3 p.o., and P at 1, lengthened survival time in KCN-treated mice.

A cardiovascular study was performed in anesthetized dogs (N=3). Increasing doses of 0.1, 0.3, and 1 mg/kg i.v. were given at 10 minute intervals. A single dog was given THA (1, 3, and 10 mg/kg). Results are shown on the following page. E produced no pronounced changes in the parameters studied. THA, in the single animal tested, produced a decreased heart rate and increased PQ interval at 3 mg/kg and above (i.e., at doses which were not tested with E).

In male rats, E at 10 mg/kg p.o. increased urine volume and urinary Na and Cl excretion over the first 4 hours post-dosing; these were followed by compensatory decreases during the 4-24 hour period such that 24 hour values were not altered (see attached figures "4", "5", and "6"). (Not shown: no effect on K excretion seen during 0-4 hour period but a decrease seen during 4-24 hour period. Also, lower doses of 1 and 3 mg/kg were tested during the 0-4 hour period and increases in urine volume and Na and Cl excretion occurred but were not statistically significant. THA at 10 mg/kg p.o. was also tested during the 0-4 hour period and produced slightly greater increases than were seen with E at 10 mg/kg). As shown in the figures, the effects of E were attenuated by scopolamine, suggesting cholinergic mediation. The report states that the attenuating effects of methscopolamine were "much weaker", suggesting a central effect of E, although the difference between the attenuating effects of scopolamine and methscopolamine appear rather slight in the figures.

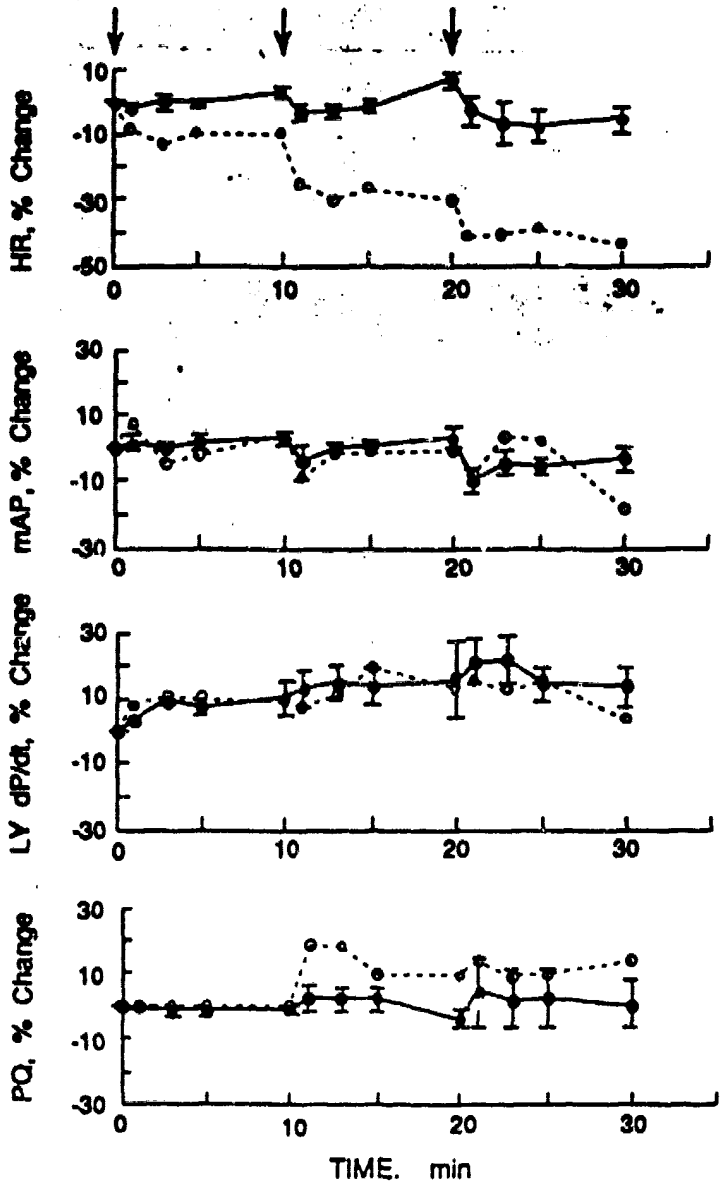

Figure 1: Effects of E2020 and THA on Cardiohemodynamics and ECG in Anesthetized Dogs. The test compounds were administered at the points indicated by the arrows at intravenous doses of 0.1, 0.3 and 1 mg/kg (E2020) and 1, 3 and 10 mg/kg (THA).

● : E2020 (n=3). ○ : THA (n=1). Each point and the bar represents mean ± S.E. HR: Heart rate, mAP: Mean arterial pressure, LV dP/dt: First derivative of left ventricular pressure, PQ: PQ-interval in ECG

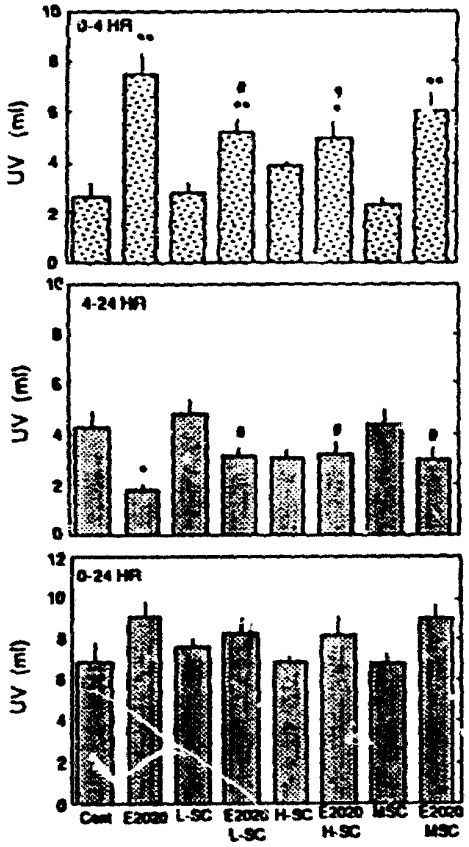

Figure 4: Effects of E2020, SC and MSC on Urinary Volume (UV)

Values represent the mean ± S.E. of 6 rats. L-SC and H-SC indicate low dose (0.5 mg/kg) and the high dose (1 mg/kg) of SC, respectively. *, **: $p < 0.05$, $p < 0.01$ vs control. #: $p < 0.05$ vs E2020 alone.

SC = Scopolamine
MSC = Methscopolamine
Dose of E2020 = 10 mg/kg p.o.

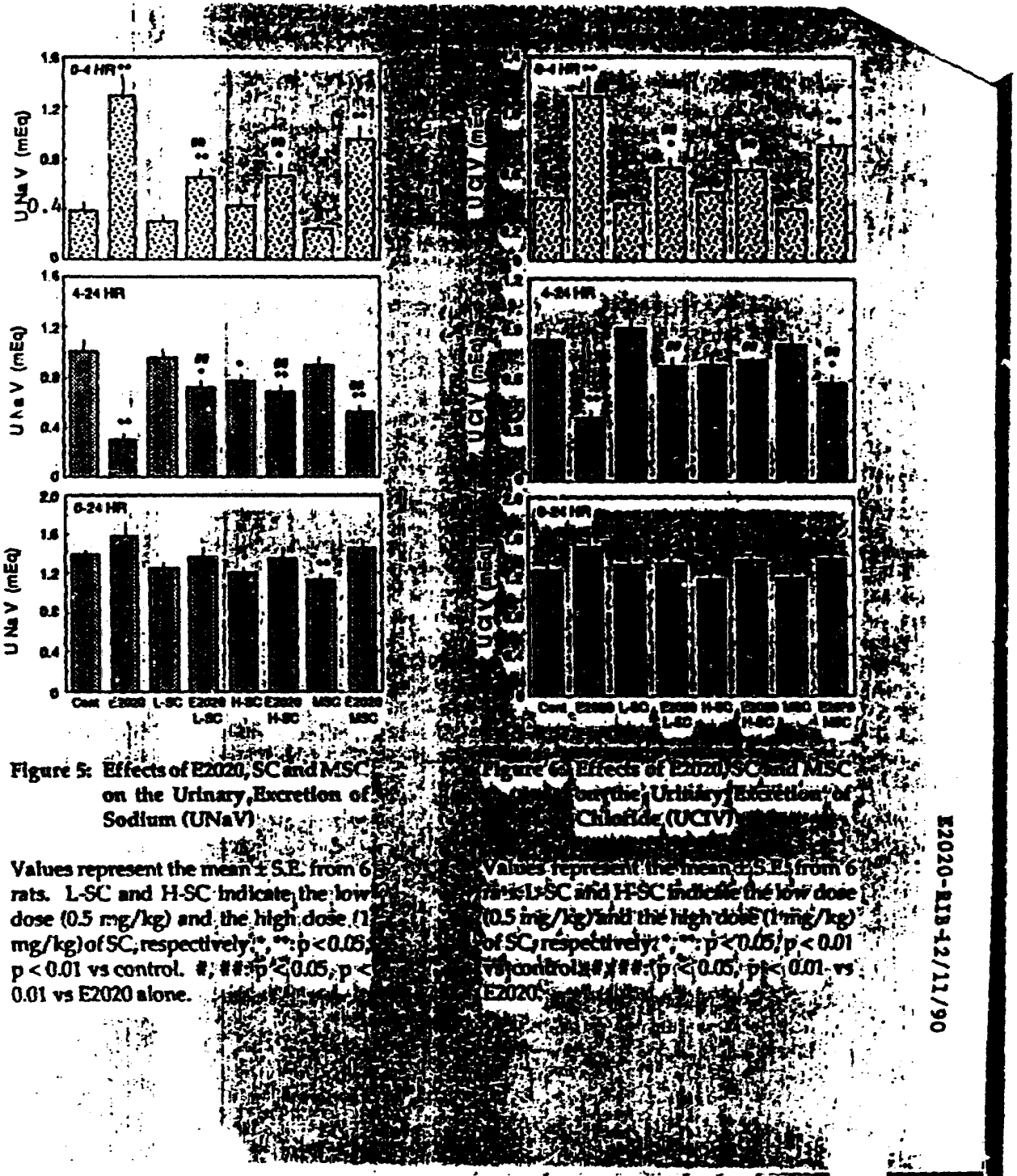

Figure 5: Effects of E2020, SC and MSC on the Urinary Excretion of Sodium (UNaV)

Values represent the mean ± S.E. from 6 rats. L-SC and H-SC indicate the low dose (0.5 mg/kg) and the high dose (1 mg/kg) of SC, respectively. *, **: $p < 0.05$, $p < 0.01$ vs control. #, ##: $p < 0.05$, $p < 0.01$ vs E2020 alone.

Figure 6: Effects of E2020, SC and MSC on the Urinary Excretion of Chloride (UCIV)

Values represent the mean ± S.E. from 6 rats. L-SC and H-SC indicate the low dose (0.5 mg/kg) and the high dose (1 mg/kg) of SC, respectively. *, **: $p < 0.05$, $p < 0.01$ vs control. #, ##: $p < 0.05$, $p < 0.01$ vs E2020.

E2020-RLB-12/11/90

In a "preliminary" study in conscious rats, intraduodenal administration of E at 1, 3, and 10 mg/kg, and THA at 3 and 10 mg/kg (only doses tested) caused dose-dependent increases in the frequency of gastric (but not duodenal) contractions. In anesthetized rats, intraduodenal administration of E at 10 but not 1 or 3 mg/kg decreased gastric acid concentration (without significant decrease in volume of gastric secretion); THA at 10 mg/kg caused a slightly smaller decrease than did E at the same dose.

E at 10 (but not 1 or 3) mg/kg p.o. elevated basal blood glucose by about 80% in fasted rats for 1-2 hour post dosing. THA produced similar effects:

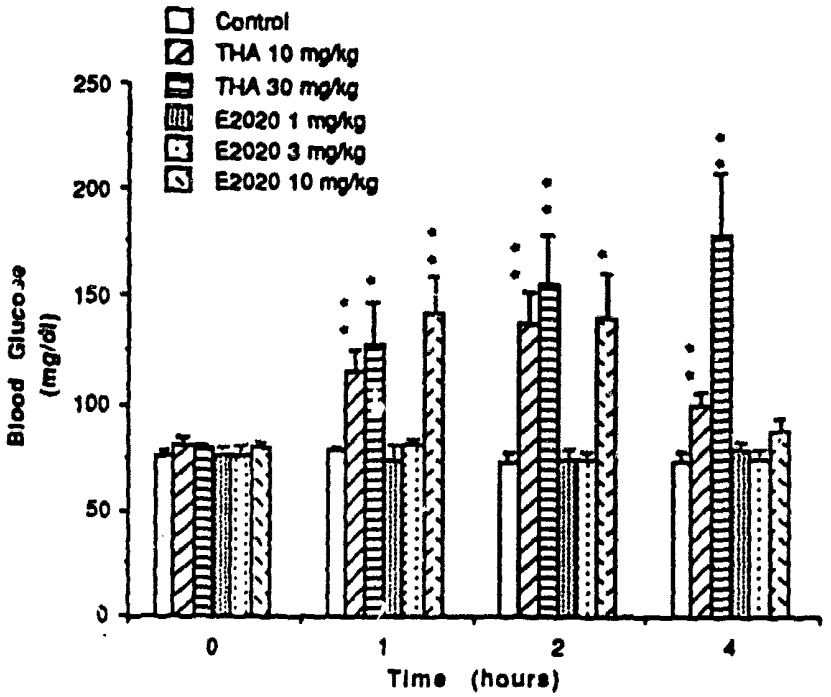

Figure 8: Basal Blood Glucose Concentrations after Administration of E2020 and THA.

Columns show mean ± S.E. (n=5 except n=4 for E2020 1 mg/kg 1, 2 and 4hr time points because of incomplete administration of E2020 solution). *, **: $p < 0.05$, 0.01 vs respective control level at each time point.

E at 10 mg/kg (but not 1-3) and THA at 10-30 mg/kg p.o. decreased glucose tolerance in fasted rats:

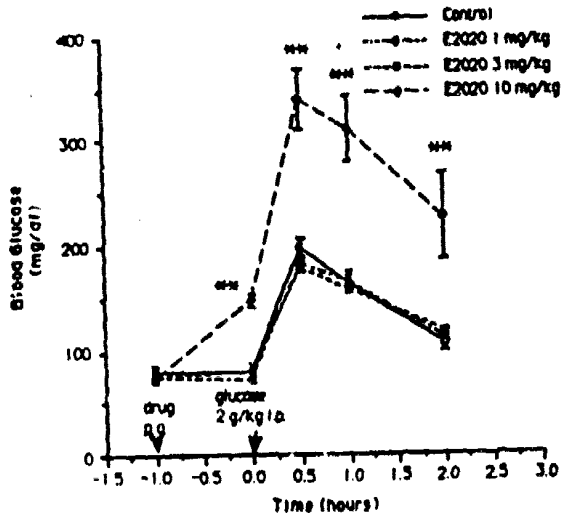

Figure 9: Effect of E2020 on Glucose Tolerance in Rats.
Mean ± S.E. n=5 except n=4 for control at 0, 0.5, 1 and 2 hr (because of incomplete administration of distilled water) and E2020 10 mg/kg at 0, 0.5, 1 and 2 hr (because of severe diarrhea). *, **: $p < 0.05$, 0.01 vs the respective control level at each time point.

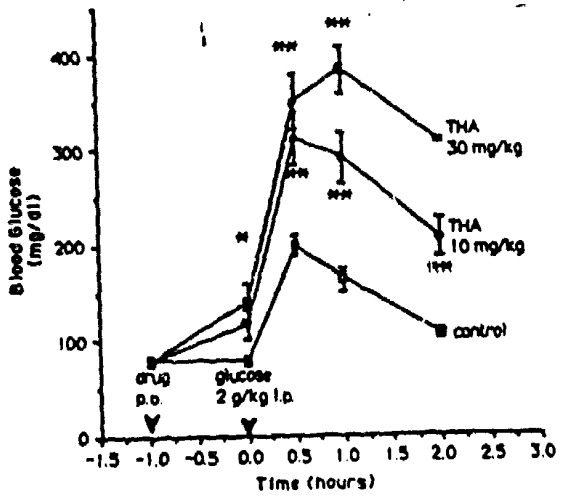

Figure 10: Effect of THA on Glucose Tolerance in Rats. Mean ± S.E. n=5 except n=4 for control at 0, 0.5, 1 and 2 hr (because of incomplete administration of distilled water), THA 10 mg/kg at 0.5, 1, 2 hr (because of severe diarrhea) and THA 30 mg/kg at 1 hr (because of the death of one rat). n=2 for THA 30 mg/kg at 2 hr (because of the death of an additional two rats). *, **: $p < 0.05$, 0.01 vs the respective control level at each time point.

E was a relatively weak inhibitor of collagen-induced platelet aggregation in human platelet-rich plasma (IC50% about 500 uM).

ADME/PK:

The sponsor's narrative summary and table of studies are attached. The following discussion involves data on metabolites in rat and dog which recently have become available. (Also note that data on plasma drug levels obtained in conjunction with toxicity studies are summarized in the individual studies in the present review).

The proposed metabolic pathway is shown in the attached figure (figure "7"). Also attached are tables ("14" and "10") showing the relative amounts of parent drug and various metabolites in plasma, urine, feces, and selected tissues after oral dosing with 1 mg/kg C^{14} - labeled E2020 in rats and dogs. Note that in both species the drug is extensively metabolized as indicated by the relatively low proportion of parent drug in plasma, urine, feces, and bile. (For interpretation of the urine and feces data, note that the % of dose excreted as total label in the time intervals specified was as follows: rat - 37% in urine and 54% in feces; dog - 20% in urine and 71% in feces). (Note that in contrast, proportions of parent drug in brain, liver, kidney, and pancreas were relatively high at the time point measured). Data showing plasma and brain levels in rats over a larger time span are shown in attached figure "5". It can be seen that in plasma, parent drug is initially ~ 1/3 total label and decreases to a much lower fraction over time. In brain, parent drug is responsible for most of the label over the time period measured. Plasma data in dog are shown in attached figure "4" where levels of parent drug are much lower than those of total label over the time period measured. (The 0-6 hr. AUC for parent drug was ~ 3% that of total label). As noted under "Pharmacodynamics", metabolites M1 and M3 had AchE inhibiting potencies in vitro similar to that of the parent compound.

(Note that until recently there were no adequate data on extent of absorption of drug in dogs. Bioavailability was known to be low. Although there is apparently still not a study using i.v. dosing with labeled drug in dogs, which would be needed to more directly estimate oral absorption, recent data showing a large amount of label found in bile after oral dosing, and the presence of only a small amount of unchanged drug in feces after oral dosing [table "10"], suggest that the low bioavailability in dogs is due to first-pass metabolism rather than poor absorption, and the relatively high amount of label excreted in feces after oral dosing represents biliary excretion rather than unabsorbed drug. [Extensive oral absorption was more conclusively demonstrated in rats in earlier studies by the use of i.v. dosing with labeled drug, and by the use of bile-cannulated animals. The relatively low amount of unchanged drug excreted in feces (4% of dose; see table "14") supports this conclusion]).

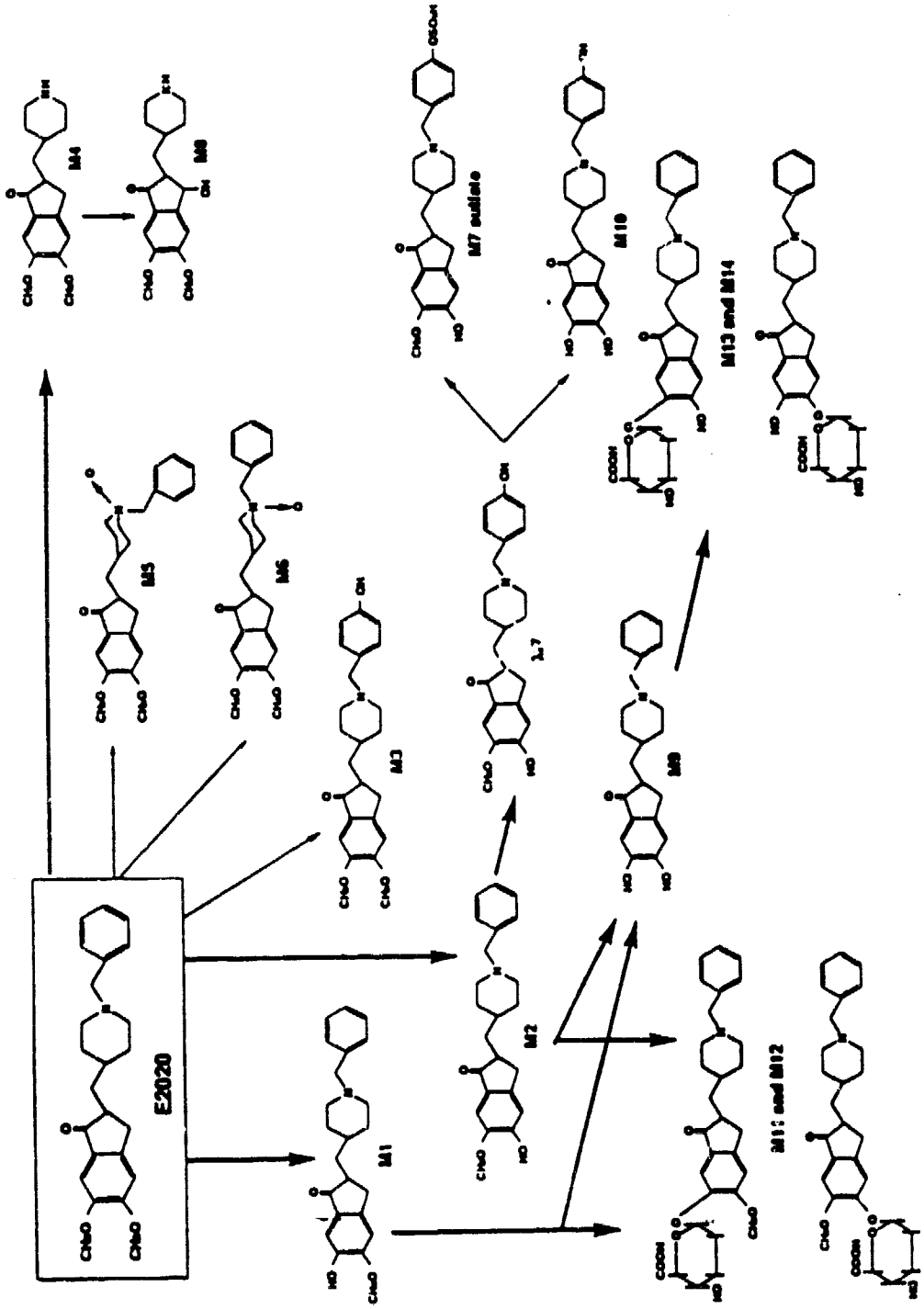

Figure 7 Proposed metabolic pathways
—→ main pathways
—— minor pathways

RAT

Table 14 Composition of metabolites in plasma, tissues and excreta after a single oral administration of ^{14}C-E2020 (1mg/kg) to rats.

	Plasma 0.5hr		Brain 0.5hr		Urine 0 - 24 hr		Feces 0 - 24 hr		Bile 0 - 24 hr		Liver 0.5hr		Kidney 0.5hr		Pancreas 0.5hr		Pancreas 8hr	
	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M	Mean	S.E.M
	(% of Sample)				(% of dose)						(% of Sample)							
E2020	31.0 ± 2.89		89.8 ± 6.18		1.52 ± 0.23		4.21 ± 0.56		0.22 ± 0.01		80.8 ± 1.49		[illegible]		95.8 ± 1.48		98.8 ± 0.27	
M1	—		—		2.48 ± 0.89		3.37 ± 1.19		0.35 ± 0.07		1.78 ± 0.29		0.9[illegible] ± 0.[illegible]		BQL		BQL	
M2	—		—		1.01 ± 0.46		1.20 ± 0.33		0.79 ± 0.03		2.10 ± 0.16		[illegible].52 ± 0.[illegible]5		0.78 ± 0.08		BQL	
M3	—		—		0.08 ± 0.02		1.33 ± 0.29		0.10 ± 0.01		0.26 ± 0.02		BQL		BQL		BQL	
M4	3.29 ± 0.83		—		3.34 ± 0.20		3.61 ± 0.30		6.80 ± 1.08		4.72 ± 0.46		8.12 ± 0.85		1.43 ± 0.23		BQL	
M5	—		—		0.47 ± 0.03		0.18 ± 0.03		0.32 ± 0.05		0.14 ± 0.01		BQL		BQL		BQL	
M6	—		—		0.06 ± 0.00		0.11 ± 0.01		0.07 ± 0.00		BQL		BQL		BQL		BQL	
M7	—		—		0.11 ± 0.03		1.10 ± 0.14		0.10 ± 0.01		0.70 ± 0.13		BQL		BQL		BQL	
M7-sulfate	—		—		1.01 ± 0.62		3.93 ± 0.77		2.13 ± 0.28		0.81 ± 0.14		BQL		—		—	
M8	—		—		0.15 ± 0.10		1.56 ± 0.31		0.96 ± 0.13		0.54 ± 0.24		BQL		—		—	
M9	—		—		0.85 ± 0.39		13.5 ± 2.39		0.64 ± 0.03		BQL		BQL		—		—	
M10	—		—		0.12 ± 0.04		1.33 ± 0.10		0.25 ± 0.02		BQL		BQL		—		—	
O-glucuronide	55.1 ± 5.33		—		23.0 ± 2.57		6.87 ± 0.78		65.7 ± 0.95		4.17 ± 0.82		15.1 ± 4.01		—		—	
UM1	—		—		0.75 ± 0.28		3.89 ± 0.26		1.20 ± 0.11		0.53 ± 0.43		BQL		—		—	
UM2	—		—		0.17 ± 0.07		1.46 ± 0.12		1.08 ± 0.13		BQL		BQL		—		—	
UM3	—		—		0.33 ± 0.04		0.88 ± 0.13		8.37 ± 0.66		0.38 ± 0.10		BQL		—		—	
Others	10.7 ± 1.37		10.2 ± 6.18		1.45 ± 0.32		5.84 ± 0.19		11.0 ± 1.38		2.93 ± 0.25		1.95 ± 0.72		1.20 ± 0.33		0.81 ± 0.37	

Mean and S.E.M. are calculated for four rats at each time point.

BQL: Below quantification limit (< 40dpm/Sample: 0.135ng equiv./Sample).

— : not measured

Study No. A91009

DOG

Table 10 Composition of metabolites in plasma, tissues and excreta after a single oral administration of ^{14}C-E2020 (1.0mg/kg) to beagle dogs.

	Plasma 1.5hr		Brain 1.5hr		Urine 0 - 48hr		Feces 0 - 48hr		Urine in urinary bladder 1.5hr		Bile 1.5hr		Liver 1.5hr		Kidney 1.5hr		Pancreas 1.5hr	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
	(% of Sample)		(% of Sample)		(% of dose)		(% of dose)		(% of Sample)		(% of Sample)		(% of Sample)		(% of Sample)		(% of Sample)	
E2020	6.21	± 0.99	80.8	± 3.86	0.32	± 0.09	0.61	± 0.19	2.31	± 0.31	1.45	± 0.51	25.8	± 2.17	39.0	± 6.61	79.2	± 4.42
M1	BQL		6.22	± 1.65	0.19	± 0.04	4.14	± 0.35	0.46	± 0.06	0.18	± 0.07	1.41	± 0.24	1.89	± 0.13	4.73	± 0.80
M2	1.32	± 0.19	6.76	± 1.84	1.07	± 0.19	12.2	± 2.04	0.59	± 0.10	0.39	± 0.12	6.16	± 0.19	5.01	± 0.46	1.63	± 0.71
M3	0.34	± 0.17	NM		0.12	± 0.03	0.33	± 0.06	0.23	± 0.04	0.08	± 0.01	3.05	± 0.20	2.63	± 0.17	1.13	± 0.08
M4	8.33	± 0.39	NM		1.56	± 0.10	6.04	± 1.01	19.6	± 2.16	31.9	± 3.39	7.41	± 0.56	15.7	± 1.51	5.58	± 1.32
M5	0.29	± 0.15	NM		0.23	± 0.08	0.47	± 0.05	2.32	± 0.38	1.36	± 0.83	0.30	± 0.18	0.26	± 0.15	0.44	± 0.16
M6	BQL		NM		0.03	± 0.00	0.10	± 0.03	0.68	± 0.28	0.08	± 0.01	0.29	± 0.03	BQL		0.12	± 0.07
M7	BQL		NM		0.40	± 0.07	4.25	± 0.38	0.53	± 0.06	0.11	± 0.00	1.16	± 0.10	0.85	± 0.18	BQL	
M7-sulfate	1.75	± 0.35	NM		2.07	± 0.19	2.79	± 0.21	1.47	± 0.51	5.06	± 0.06	1.15	± 0.15	1.25	± 0.22	1.98	± 0.58
M8	1.78	± 0.50	NM		0.22	± 0.03	3.23	± 0.35	5.66	± 0.45	7.39	± 0.32	2.74	± 0.15	2.80	± 0.70	2.63	± 0.52
M9	BQL		NM		0.06	± 0.01	1.14	± 0.21	3.67	± 0.77	1.98	± 0.43	0.63	± 0.06	0.35	± 0.13	NM	
M10	BQL		NM		0.03	± 0.01	0.57	± 0.02	1.06	± 0.28	0.30	± 0.07	0.41	± 0.07	0.13	± 0.07	NM	
O-glucuronide	55.1	± 0.57	NM		8.56	± 0.87	6.15	± 1.14	46.7	± 3.49	31.7	± 1.63	22.2	± 0.23	16.5	± 3.77	NM	
UM1	2.42	± 0.14	NM		0.31	± 0.02	2.74	± 0.49	1.95	± 0.02	0.77	± 0.07	2.01	± 0.48	1.29	± 0.21	NM	
UM2	1.68	± 0.09	NM		0.86	± 0.14	13.6	± 1.97	5.97	± 1.26	7.56	± 1.26	3.73	± 0.86	1.52	± 0.36	NM	
UM3	16.2	± 1.23	NM		2.45	± 0.45	1.00	± 0.06	2.93	± 0.13	1.25	± 0.06	3.18	± 0.13	1.76	± 0.41	NM	
Others	3.85	± 0.83	6.19	± 1.09	1.27	± 0.16	10.9	± 1.42	3.89	± 0.73	8.40	± 1.77	17.5	± 1.85	9.02	± 0.87	2.56	± 0.87

Mean and S.E.M. are calculated for three dogs at each time point.
BQL: Below quantification limit (< 40dpm/sample:0.136ng-equiv./sample).
NM: not measured.

RAT

Study No. A91009

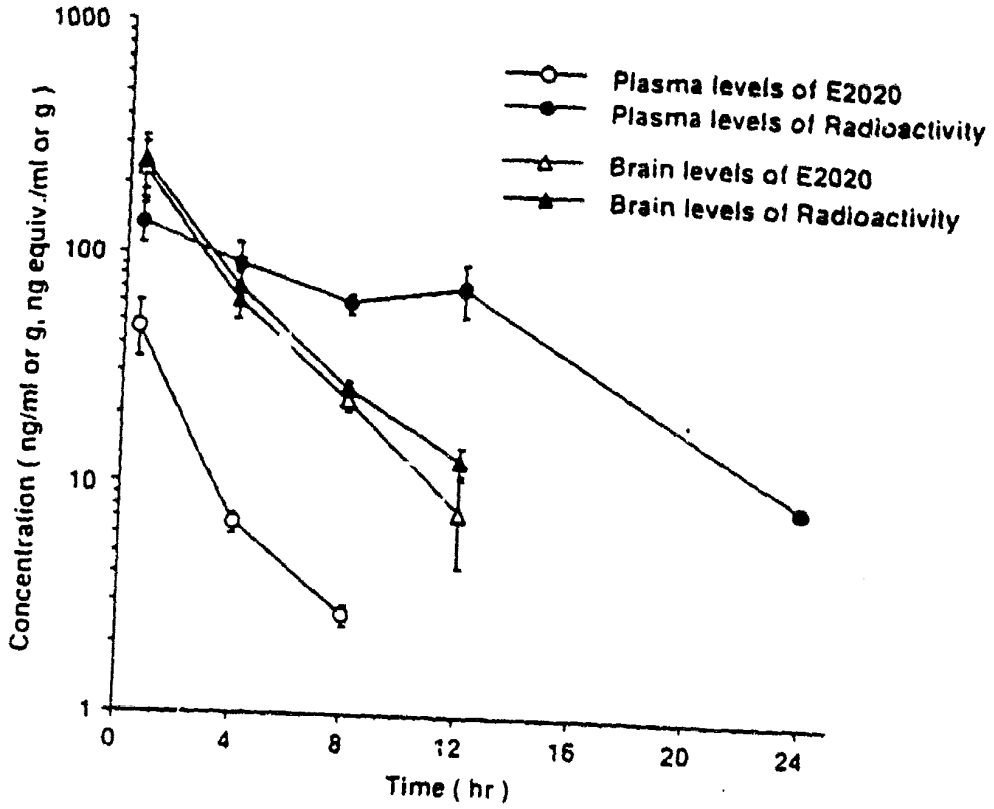

Figure 5 Plasma and brain levels of E2020 and radioactivity after a single oral administration of ^{14}C-E2020 (1mg/kg) to rats.
Each point represents the mean with S.E.M. of four animals.

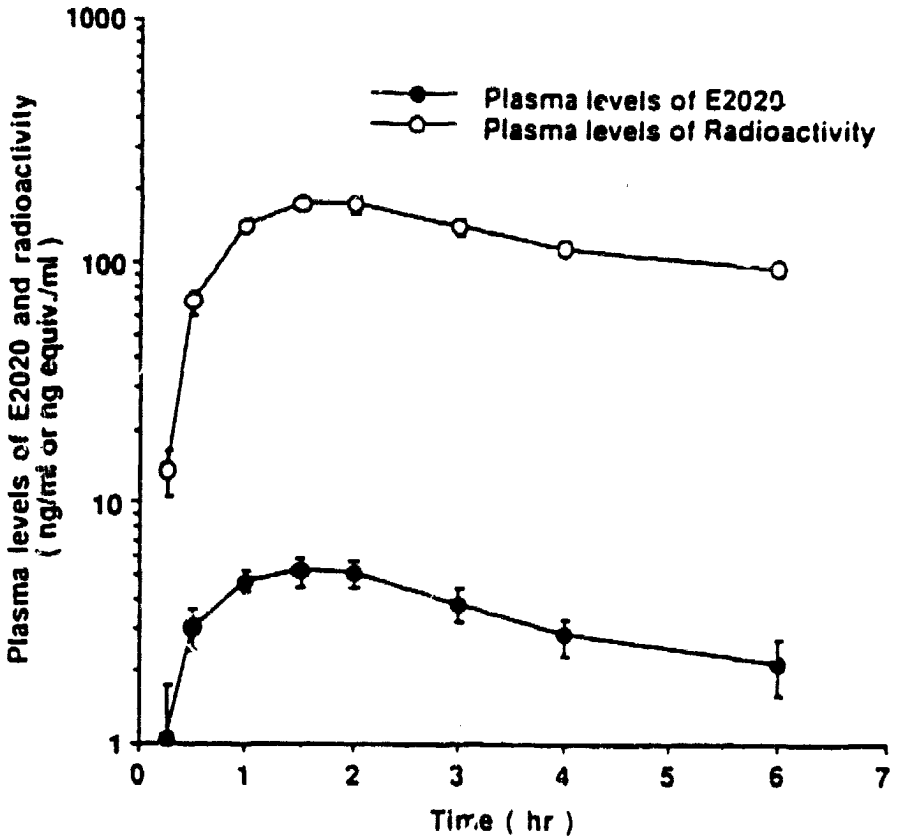

Figure 4 **Plasma levels of E2020 and radioactivity after a single oral administration of ^{14}C-E2020 (1.0mg/kg) to beagle dogs.**
Each point represents the mean with S.E.M. of three animals.

SPONSOR'S SUMMARY TABLE OF STUDIES (ADME/PK)

E2020: NONCLINICAL ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION (ADME) STUDIES

Table of Studies

(Section 5 . D . 2 . 0)

Study Ref.	Title of Study:	Pdf Report Location	Species/ Strain	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	$t_{1/2}$[e] (hr)	GLP Status; Laboratory
B-1[d]	Plasma, Brain, and Liver Levels of E2020 After Oral and Intravenous Administration of Single Doses to Rats. (Study No. A00001; Report No. W-931117)	Volume 1.046 Page 1	Rat (Sprague)	Male	4/ timepoint	p.o. (water)	1.0	E2020	plasma	43.3	0.5	119[h]	6.38	GLP; Hisai Institute of Drug Metabolism and Pharmacokinetics, Tsukuba, Japan
							3.0			119	0.5	265[h]	3.68	
							10			638	0.5	1840[h]	4.98	
						i.v. (saline)	3.0			345	(0.083)	614[h]	3.88	
						p.o. (water)	1.0	E2020	brain	259	0.5	988[h]	6.68	
							3.0			892	0.5	2130[h]	3.49	
							10			5620	0.5	13360[h]	4.78	
						i.v. (saline)	3.0			5790	(0.083)	4730[h]	2.67	
						p.o. (water)	1.0	E2020	liver	7080	0.25	13480[h]	5.59	
							3.0			19480	0.25	29180[h]	3.89	
							10			69080	0.25	100000[h]	5.44	
						i.v. (saline)	3.0			8390	(0.083)	26260[h]	5.81	

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.
† Second maxima (seen in profiles of radioactivity of intact, but not bile-duct cannulated rats) at 4 - 6 hr after dosing.
[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.
[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.
[c] AUC = Area under curve, determined by trapezoide to infinite time (∞) or last quantifiable concentration (t): [g] t=72 hr; [h] t=24hr; [d] t= 8hr; [i] t= 6hr; [k] t= -.
[e] $t_{1/2}$ = Apparent half-life, calculated from 0.693/J, where J is the slope of the elimination phase in the log, transformed concentration versus time curve.
[f] =Information previously submitted; complete report submitted for first time in NDA. [d]=Not previously submitted.

Study Ref.	Title of Study:	Full Report Location	Species/ Strain	Gender	Number of Animals	Route of Admin. (vehicle)	E2070 Dose (mg/kg)	Compound Measured	Matrix	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng·hr/ml)	$t_{1/2}$[e] (hr)	GLP Status; Lab country
D-5[g]	The Pharmacokinetic Monitoring of E2020 After a Single Oral Administration of E2020 to Rats (Study No. 930512; Report No. W-19930220)	Volume 1.046 Page 48	Rat/ (non-fasting)	Male	4/ group	p.o. (water)	1.0 3.0 10	E2020	plasma	5.45 19.6 71.8	1.0 0.5 0.5	9.8[d] 65.6[d] 269[d]	NR	GLP; Eisai Drug Metabolism Research Station, Tsukuba, Japan
				Female	4/ group		1.0 3.0 10	E2020	plasma	14.7 46.9 152	1.0 0.75 0.75	54.1[d] 208[d] 597[d]	NR	

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.
† Second maximum (seen in profiles of radioactivity of intact, but not bile-duct cannulated rats) at 4 - 6 hr after dosing.
[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.
[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.
[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [g] t = 72 hr; [h] t = 24hr; [d] t = 8hr; [f] t = 6hr; [e] t = ∞.
[e] $t_{1/2}$ = Apparent half-life, calculated from 0.693/J, where J is the slope of the elimination phase in the log, transformed concentration versus time curve.
[f] = Information previously submitted; complete report submitted for first time in NDA. [g] = Not previously submitted.

Study Ref.	Title of Study:	Full Report Location	Species/ Status	Gender	Number of Animals	Route of Admin. (vehicle)	Dose (mg/kg)	Compound Measured	Matrix	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	$t_{1/2}$[d] (hr)	GLP Status; Laboratory
D-3[e]	[illegible]	Volume 1.046 Page 82	Rat/ (fasting)	male	3	p.o. (0.01 M HCl)	1.0 (33 mCi/ mmol)	^{14}C	tissues	NR	NR	NR	NR	

Values shown are the mean parameter, except for Tmax which is the median.
NR = not recorded in this study.
† Second maximum (seen in profiles of radioactivity of intact, but not bile-duct cannulated rats) at 4 - 6 hr after dosing.
[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.
[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.
[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [g] t = 72 hr; [h] t = 24hr; [i] t = 8hr; [j] t = 6hr; [k] t = ∞.
[d] $t_{1/2}$ = Apparent half-life, calculated from 0.693/j, where j is the slope of the elimination phase in the $\ln_e$ transformed concentration versus time curve.
[e] = Information previously submitted; complete report submitted for first time in NDA. [n] = Not previously submitted.

Section 5-Non Clinical Pharmacology and Toxicology

III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Strain	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	$t_{1/2}$[d] (hr)	GLP Status; Laboratory
B-4[f]	Absorption, Distribution, Metabolism and Excretion of ^{14}C-E2020 After a Single Oral Administration to Rats. (Study No. A91089; Report No. W-93110)[f]	Volume 1.946 Page 133	Rat/Intact Rat/Bile cann. (fasting)	Male	4 (3)o /group	p.o. (water)	1.0 133.54 (µCi/mg)	^{14}C ^{14}C	blood blood	61.1† 107	0.5† 1	1346[g] 657[g]	NR NR	GLP; Biosi Section of Drug Metabolism and Pharmacokinetics, Tsukuba, Japan
			Rat/Intact	Male			1.0	^{14}C E2020 ^{14}C E2020	plasma brain	136 47.5 232 234	0.5 0.5 0.5 0.5	NR	NR	
B-5[f]	Blood Concentrations, Distribution, Metabolism, and Excretion of ^{14}C-Labelled E2020 After a Single Intravenous Administration to Rats. (Report No. W-900824)	Volume 1.946 Page 194	Rat/Intact Rat/Bile cann. (non-fasting)	Male	3 (2)o /group	i.v. (water)	1.0 107.31 (µCi/mg)	^{14}C ^{14}C	blood blood	288† 245	(0.05)† 0.083	1382[h] 989[h]	NR NR	Non-GLP; Biosi Section of Drug Metabolism and Pharmacokinetics, Tsukuba, Japan

Values shown are the mean parameters, except for Tmax which is the median.

NR = not recorded in this study.

o = one bile-cannulated rat excluded from part of the data collection/analysis.

† Second maxima (seen in profiles of radioactivity of intact, but not bile-duct cannulated rats) at 4 - 6 hr after dosing.

[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.

[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.

[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [g] t = 72 hr; [h] t = 24hr; [i] t = 8hr; [j] t = 6hr; [k] t = ∞.

[d] $t_{1/2}$ = Apparent half-life, calculated from $0.693/\int$, where $\int$ is the slope of the elimination phase in the $\log_e$ transformed concentration versus time curve.

[f] = Information previously submitted; complete report submitted for first time in NDA. [g] = Not previously submitted.

Section 5-Non Clinical Pharmacology and Toxicology

III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Strain	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	$t_{1/2}$[e] (hr)	GLP Status; Laboratory
B-6[d]	Plasma Levels of E2020 After Oral and Intravenous Administration of Single Doses to Beagle Dogs. (Study No. A91006; Report No. W-931118)	Volume 1.046 Page 227	Beagle/ dog (fasting)	Male	6 (3 way X-over design	p.o. (water in capsule)	1.0	E2020	plasma	3.37	1.0	10.9[k]	NR	GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan
							3.0			49.5	1.3	159[k]	3.28	
										168	(8.083)	192[k]	3.04	
						i.v. (saline)	1.0							
B-7[d]	Absorption, Distribution, Metabolism and Excretion of ^{14}C-E2020 After a Single Oral Administration to Beagle Dogs. (Study No. A92002; Report No. W-931120)	Volume 1.046 Page 270	Beagle/ dog (fasting)	Male	3/ group	p.o. (water in capsule)	1.0	^{14}C E2020	plasma	181	1.5	734[j]	NR	GLP; Eisai Section of Drug Metabolism and Pharmacokinetics, Tsukuba, Japan
										5.46	1.5	28.4[j]	3.65	
							1.0	^{14}C	blood	123	1.5	2366[k]	98.7	
									plasma	181	1.5	3508[k]	109	
									erythrocyte	36.6	1.5	504[k]	NR	

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.

† Second maxima (seen in profiles of radioactivity of intact, but not bile-duct cannulated rats) at 4 - 6 hr after dosing.

[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.

[b] Tmax = Time of peak concentration *vs* (first sample collected), determined by inspection of the data.

[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [g] t = 72 hr; [h] t = 24hr; [i] t = 8hr; [j] t = 6hr; [k] t = ∞.

[e] $t_{1/2}$ = Apparent half-life, calculated from 0.693/J, where J is the slope of the elimination phase in the log, transformed concentration versus time curve.

[d] = Information previously submitted; complete report submitted for first time in NDA. [#] = Not previously submitted.

Section 5-Non Clinical Pharmacology and Toxicology

III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Status	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Study Day	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	GLP Status; Laboratory
B-8[d]	The Pharmacokinetic Monitoring of E2020 During Repeated Oral Administration to Rats. (Study No. 930513; Report No. W-19952221)	Volume 1.047 Page 1 †	Rat (non-fasted)	Male	4/group	p.o. (water)	1.0	E2020	plasma	1	8.43	0.5	22.8[d]	GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan
							3.0				18.7	1.25	73.9	
							10				59.3	0.5	291	
							1.0			15	5.59	2.0	25.1	
							3.0				18.6	2.0	79.0	
							10				91.2	2.0	476	
				Female			1.0	E2020	plasma	1	16.9	1.25	60.7[d]	
							3.0				67.8	0.5	270	
							10				147	0.5	785	
							1.0			15	13.2	2.0	63.2	
							3.0				68.9	2.0	337	
							10				291	0.5	1225	

Values shown are the mean parameters, except for Tmax which is the median.

† Double maxima at ≤1 h and 4 -10h.

[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.

[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.

[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [h] t = 24hr; [d] t = 8hr; [i] t = 10 hr.

[d] = Information previously submitted; complete report submitted for first time in NDA. [e] = Not previously submitted.

Study Ref.	Title of Study:	Full Report Location	Species\ Status	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Study Day	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	GLP Status; Laboratory
B-9[d]	Plasma Concentration Measurements in Chronic Toxicity Study of E2020 in Rats. (Study No. G91-2032; Report No.89-3499)	Volume 1.047 Page 58	Rat (non-fasted)	Male	n = 5/sex/ group	p.o. (water)	1 3 10 1 3 10	E2020	plasma	1 361 	7.42 24.1 61.7 19.9 87.4 234	[1.0] [1.0] [1.0] [1.0] [1.0] [1.0]	NR	
				Female			1 3 10 1 3 10	E2020	plasma	1 361 	18.0 57.1 85.7 23.9 101 242	[1.0] [1.0] [1.0] [1.0] [1.0] [1.0]	NR	

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.
†Double maximas ≤ 1h and at 4 -10h.
[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.
[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.
[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [h] t = 21hr; [d] t = 8hr, [i] t = 10 hr.
[d] = Information previously submitted; complete report submitted for first time in NDA. [e] = Not previously submitted.

Section 5-Non Clinical Pharmacology and Toxicology

III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Status	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Study Day	Cmax[a] (ng/ml)	Tmax[b]	AUC[c] (ng.hr/ml)	GLP Status; Laboratory
D-10[e]	13-Week Toxicity and Toxicokinetic Study of E2020 in Rats by Dietary Administration. (Study No. HWA-2656100)	Volume 1.047 Page 111	Rat	Male	4/ timepoint	p.o. (diet)	15	E2020	plasma	1	105	03.00[f]	1713[h]	
							30				238	00.00	3190	
							60				810	03.00	10170	
							15			85	62.4	[03.00]	NR	
							30				166	[00.00]	NR	
							60				426	[03.00]	NR	
				Female			15	E2020	plasma	1	241	03.00[f]	3215[h]	
							30				574	00.00	7663	
							60				1379	06.00	19939	
							15			85	193	[03.00]	NR	
							30				557	[00.00]	NR	
							60				1096	[06.00]	NR	
D-11[e]	Absorption, Distribution and Excretion of ^{14}C-Labeled E2020 After Repeated Oral Administration to Rats. (Report No. W-890820)	Volume 1.047 Page 112	Rat (non-fasted)	Male	3-4/ group	p.o. (water)	1.14	^{14}C	blood	1 7	56† 60†	0.72† 0.82†	479.5[i] 585.0	Non-GLP; Eisai Drug Metabolism & Pharmacokinetic Unit, Tsukuba, Japan

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.

† Double maxima at ≤1 h and 4 - 10 hr.

[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.

[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.

[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [h] t = 21 hr; [i] t = 8hr; [j] t = 10 hr.

[f] 24 hour clock time.

[e] = Information previously submitted; complete report submitted for first time in NDA. [#] = Not previously submitted.

Section 5-Non Clinical Pharmacology and Toxicology

III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Strain	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Study Day	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	GLP Status; Laboratory
3-12[B]	Absorption, Distribution, Metabolism and Excretion Studies in Rats After Single and Repeated Oral Administration. (Study No. ESI 110C/ 950263; Report No. W-19952855)	Volume 1.047 Page 134	Rat	Male (non-fasted)	3/group	PO (0.01 M HCl)	1.0	^{14}C	blood	1 / 14	63.7† 87.4†	5.0† 6.7†	796.2[h] 1081[h]	-
				Female (pregnant)			1.0	^{14}C	blood	GD 7 GD 19	83.7 63.0	(0.5) 8.0†	NR	

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.

†Double maxima ≤ 1 h and 4 - 10 hr.

[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.

[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.

[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [h] t = 24hr; [d] t = 8hr; [i] t = 10 hr.

[f] =information previously submitted; complete report submitted for first time in NDA. [B] =Not previously submitted.

Study Ref.	Title of Study:	Full Report Location	Species\ Strain	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Component Measured	Matrix	Study Day	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	GLP Status; Laboratory
D-15[j]	13-Week Toxicity and Toxicokinetic Study of E2020 in Mice by Dietary Administration. (Study No. HWA-2656101)	Volume 1.047 Page 273	Mouse	Male	4/ timepoint	p.o. (diet)	15	E2020	plasma	15	11.78	00.00[f]	129.5[h]	
							30				34.06	00.00	441.6	
							60				195.0	00.00	2310	
							90				311.0	00.00	4623	
							15			85	21.76	[00.00]	NR	
							30				50.15	[00.00]	NR	
							60				131.2	[00.00]	NR	
							90				283.5	[00.00]	NR	
				Female			15	E2020	plasma	15	13.40	00.00[f]	125.3[h]	
							30				35.52	03.00	346.4	
							60				114.7	00.00	1337	
							90				207.7	03.00	3120	
							15			85	21.55	[00.00]	NR	
							30				26.57	[00.00]	NR	
							60				86.48	[00.00]	NR	
							90				275.0	[00.00]	NR	

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.

[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.

[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.

[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [h] t = 21hr; [d] t = 8hr; [i] t = 6hr.

[f] =Information previously submitted; complete report submitted for first time in NDA. [#] =Not previously submitted.

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III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Status	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Study Day	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	GLP Status; Laboratory
B-14[f]	Plasma Levels of E2020 During and After Repeated Oral Administration to Beagle Dogs for Thirteen Weeks (Study No. 882142; Report No. W-900939)	Volume 1.047 Page 274	Dog/ (non-fasting)	Male Female	3/group	p.o. (powder in capsule)	1.0	E2020	plasma	1 35 91	4.0 6.6 8.9	1.0 2.0 2.0	11.8[d] 20.3[d] 26.6[d]	GLP; Eisai Drug Metabolism & Pharmacokinetic Unit, Tsukuba, Japan
							3.0	E2020	plasma	1 35 91	54 154 153	1.0 1.0 1.5	140 598 550	

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.

[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.

[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.

[c] AUC = Area under curve, determined by trapezoids to infinite time (∞) or last quantifiable concentration (t): [d] t = 24hr; [e] t = 8hr; [h] t = 10hr.

[f] = Information previously submitted; complete report submitted for first time in NDA. [*] = Not previously submitted.

Study Ref.	Title of Study:	Full Report Location	Species/ Strain	Gender	Number of Animals	Route of Admin. (vehicle)	E2020 Dose (mg/kg)	Compound Measured	Matrix	Study Day	Cmax[a] (ng/ml)	Tmax[b] (hr)	AUC[c] (ng.hr/ml)	GLP Status; Laboratory
D-15[f]	Toxicokinetic Parameters in Chronic Toxicity Study of E2020 in Dogs. (Study No. G91-3033; Report No.89-3498)	Volume 1.047 Page 311	Dog (non-fasting)	Male Female	3/sex/ group	p.o. (powder in capsule)	0.6	E2020	plasma	1	3.76	2.5	11.79[h]	GLP; Eisai Department of Drug Safety Research, Kawashima, Japan
										28	5.99	1.0	20.62[h]	
										182	7.18	1.5	23.65[h]	
										364	8.02	2.0	39.54[h]	
							2.0	E2020	plasma	1	19.48	1.5	69.71[h]	
										28	40.88	1.5	248.7[h]	
										182	52.37	1.5	310.3[h]	
										364	59.80	2.5	404.3[h]	
							5.0	E2020	plasma	1	90.44	1.5	538.3[h]	
										28	246.9	1.5	1927[h]	
										182	298.7	1.5	2740[h]	
										364	303.1	2.0	3336[h]	

Values shown are the mean parameters, except for Tmax which is the median. NR = not recorded in this study.

[a] Cmax = Peak drug concentration or total radioactivity concentration in plasma (ng/ml or ng-eq./ml) or tissue (ng/g or ng-eq./g), determined by inspection of the data.

[b] Tmax = Time of peak concentration or (first sample collected), determined by inspection of the data.

[c] AUC = Area under curve, determined by trapezoids to infinite time (~) ... last quantifiable concentration (t): [h] t = 24hr; [d] t = 8hr; [i] t = 10hr.

[f] =Information previously submitted; complete report submitted for first time in NDA. [#] =Not previously submitted.

Section 5-Non Clinical Pharmacology and Toxicology

III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Strains	Gender	Number of Animals (vehicle)	Route of Admin.	E2020 Dose (mg/kg)	Duration	Matrix	Observations	GLP Status; Laboratory
D-16 [a]	The Protein Binding of E2020 in Rat, Dog, and Human Plasma (Study No. 910901; Report No. W-19960419)	Volume 1.047 Page 352	Rat Dog Man (*in vitro*)	Male	3/species	NA	17 - 580 ng/ml*	NA	plasma	In rat plasma, protein binding was 65.96, 64.44 and 73.63% at 28, 112.4 and 579.9 ng/ml, respectively. In dog plasma, protein binding was 86.9, 81.91 and 82.85% at 26.8, 104.4 and 565.9 ng/ml, respectively. In human plasma, the protein binding was highest, mean values of 88.38, 91.20 and 89.85% at 17, 70.1 and 351.4 ng/ml.	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan
D-17 [a]	The Binding of E2020 to Isolate Albumin and α 1-Acid Glycoprotein from Human, Dog and Rat: Determination of Binding Parameters (Study No. 951025; Report No. W-19952677)	Volume 1.048 Page 1	Rat Dog Man (*in vitro*)	NR	pool	NA	[0.5-1000 μM]*	NA	albumin α1-acid glycoprotein	The total percentage bound was independent of the E2020 concentration in all three species (76-77%, 52-55%, and 60-61% for human, dog and rat, respectively). At a Cf, unbound concentration, for human plasma of 0.01 μM, the binding to albumin (65.5%) was calculated to be six times higher than that to α-acid glycoprotein (11.1%).	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan
D-18 [a]	Interaction Between E2020 and Other Drugs in the Binding to Human Albumin (Study No. 950119, Report No. W-19952681)	Volume 1.048 Page 46	Man (*in vitro*)	NR	pool	NA	[0.0, 0.3, 1, 10 μg/ml]*	NA	albumin	E2020 does not affect the binding of furosemide, digoxin, or warfarin to human albumin, and that the binding of E2020 to human albumin is not affected by furosemide, digoxin, or warfarin.	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan
D-19 [a]	Isolation and Identification of Metabolites of E2020 in Rats. (Study No. 910125; W-19952790)	Volume 1.048 Page 87	Rat	Male	10 to 8	p.o. (water)	20 on Study Day 1; then 15	daily x 7 days	urine and feces	E2020 major metabolic pathways involved O-demethylation followed by glucuronide conjugation, aromatic hydroxylation followed by sulfate conjugation, N-dealkylation and N-oxidation.	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan

* = Concentration for in vitro studies NA = Not applicable
NR = Not recorded Cl_{int} = Intrinsic clearance calculated as the ratio of V_{max} to Km.
[a] = Not previously submitted.

Section 5-Non Clinical Pharmacology and Toxicology

III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Strain	Gender	Number of Animals	Route of Admin.	E2020 Dose (mg/kg)	Duration	Matrix	Observation	GLP Status; Laboratory
D-20 *	Isolation and Identification of a Major Metabolite of E2020 formed by Beagle Dog Microsomes. (Study No. 880831; Report No. 19932789)	Volume 1.048 Page 153	Dog	Male	NA	NA	62.5 μM*	NA	microsomes	The single main metabolite in beagle dog was identified as N-oxide-E2020.	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan
D-21*	Metabolism of E2020 in Rat, Dog and Human Liver Microsomes. (Study No. 940901; Report No. W-19932791)	Volume 1.048 Page 180	Rat Dog Human	Male + female male Men	3/sex 3 1 pool	NA	1.5 - 100 μM*	NA	microsomes	In both male and female rat, the pattern of metabolite formation followed Michaelis-Menten kinetics, and the major metabolites were M1 and M2 and M4; M3 was also formed by female microsomes. The rate of metabolism in male microsomes were greater than the rate in female. In dog liver microsomes, except for the formation of M5 (N-oxide), the profile of metabolites were similar to the rat. In human microsomes, M4 was the major metabolite, but metabolism was not as extensive as in rat and dog liver microsomes. The Cl_{int} of E2020 in male rat, female rat, dog and human were 53.7±2.25, 12.6±1.61, 37.0±2.55 and 6.35 μL/min/mg of microsomal protein, respectively.	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan

* = Concentration for in vitro studies
NA = Not applicable NR = Not recorded.
[2] = Not previously submitted.

Section 5-Non Clinical Pharmacology and Toxicology

III. Absorption, Distribution, Metabolism, Excretion (ADME) Studies

Study Ref.	Title of Study:	Full Report Location	Species/ Strain	Gender	Number of Animals	Route of Admin.	E2020 Dose (mg/kg)	Duration	Matrix	Observations	GLP Status; Laboratory
B-22*	Identification of Cytochrome P450 Involved in the Metabolism of E2020 (Study No. 941101; Report No. 19952792)	Volume 1.048 Page 233	Human	NR	1 pool	NA	2-100 μM*		microsomes	The major human metabolite of E2020 in vitro, M4, is formed mainly by CYP3A4 and to a lesser extent by CYP2D6 (M1 and M2).	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan
B-23*	Interaction of E2020 with Cytochrome P450	Volume 1.048 Page 273	Human	NR	1 pool	N/A	0-91μM*		microsomes	The IC_{50} values for the interaction of E2020 with the five major drug metabolizing isoforms CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A were all greater than 100μM. The mean Ki values for the interaction of E2020 with CYP3A4 and CYP2D6 were 131 and 47 μM, respectively. Both interactions were characterized as competitive inhibition.	Non-GLP Pfizer Central Research Drug Metabolism, Groton, CT

* = Concentration for in vitro studies
NA = Not applicable
NR = Not recorded
* = Not previously submitted

Study Ref.	Title of Study:	Full Report Location	Species/ Strains	Gender	Number of Animals	Route of Admin.	E2020 Dose (mg/kg)	Duration	Matrix	Observation	GLP Status; Laboratory
B-24[a]	In Vitro E2020 Drug Interaction Study in Human Liver Microsomes (1). (Study No. 941201; Report No. 19952793)	Volume 1.048 Page 311	Human	NR	1 pool	NA	10 - 100 μM[a]	NA	microsomes	E2020 metabolite formation of M1, M2 and M4 was inhibited by cimetidine in a low affinity non-competitive manner, the IC/Km ratio for the formation was 2.87, 6.18, and 3.76. At concentrations up to 400μM, theophylline did not inhibit the formation of M1, M2, and M4. Erythromycin was shown to be a low affinity competitive inhibitor of M1, M2 and M4. Quinidine was a competitive inhibitor of M1 and M4 formation and would be expected to inhibit the metabolism of E2020 in vivo, with IC/Km ratios of M1 to M4 formation were 0.46 and 0.44, respectively. Ketoconazole was a high affinity competitive inhibitor of M1 formation and a non-competitive inhibitor of M2 and M4.	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan
B-25[a]	In Vitro E2020 Drug Interaction Study in Human Liver Microsomes (2) (Study No. 950626; Report No. 19952885).	Volume 1.048 Page 362	Human	NR	1 pool	NA	5μM[a]	NA	microsomes	The metabolism of E2020 is mainly mediated by CYP3A4 (ketoconazole) and to a lesser extent by CYP2D6 (quinidine), and little contribution is expected from the other P450 isoenzymes.	Non-GLP; Eisai Drug Metabolism Research Section, Tsukuba, Japan

[a] = Concentration for in vitro studies
NA = Not applicable NR = Not recorded
[a] = Not previously submitted

5-1

SPONSOR'S SUMMARY

5.D.3.0 SUMMARY

3.1 OVERVIEW

From the results of the preclinical pharmacokinetic studies conducted to date, the following conclusions can be drawn:

3.1.1 Absorption and Pharmacokinetics of Unlabelled E2020 (Single- and Multiple-Dose). Following oral dosing, E2020 was rapidly absorbed in Sprague-Dawley rats and in beagle dogs (T_{max} 0.5 to 2 hours).

3.1.1.1 Following single intravenous administration, the elimination half-life in rats and dogs was about 3 hours. The volumes of distribution were large (≥ 10 L/kg), indicating extensive uptake into tissues.

3.1.1.2 In fasted rats, when one group was dosed intravenously at 3.0 mg/kg and three other groups were dosed orally at 1.0, 3.0 and 10 mg/kg, absolute bioavailabilities of 58.1, 42.8, and 89.9 %, respectively, were calculated. The oral C_{max} and AUC values showed more than dose-proportional increases at 10 mg/kg, suggesting a saturable first-pass effect.

3.1.1.3 When the same oral doses were administered to rats that were allowed continuous access to food lower plasma concentrations were achieved, and the apparent saturability in the pharmacokinetics of E2020 was much less evident. C_{max} values in fasted rats were 6.1 to 8.9 times higher than in non-fasted animals of the same sex and dose group. For example, C_{max} at 3.0 mg/kg was 119 ng/ml in fasted males and 19.6 in non-fasted males. In the non-fasted animals, the 3.33 fold increment in the dose from 3.0 to 10 mg/kg produced a 3.66 and 4.14 fold increase in the mean C_{max} and $AUC_{(0\text{-}8hr)}$ values, respectively.

3.1.1.4 In a single-dose study in fasted male dogs, an i.v. dose of 1.0 mg/kg and oral doses of 1.0 and 3.0 mg/kg indicated absolute bioavailabilities of 5.85 ± 0.99 and 28.4 ± 3.0 %, respectively, for the oral doses. The mean Cmax for the oral doses were 3.37 ± 0.58 and 49.5 ± 3.61 ng/ml, respectively, with $AUC_{(0\text{-}\infty)}$ values of 10.9 ± 1.55 and 159 ± 12.6 ng•hr/ml. Thus the 3-fold increment in the

doses resulted in an increase of over 14-fold in both parameters.

3.1.1.5 When oral E2020 was administered once-daily to rats in a 1-year study and to beagle dogs in studies of 13-weeks' and 1-year's duration, steady state plasma concentrations were greater than expected for a compound with a half-life of 3 hours, and small but progressive increases in C_{max} and AUC were observed. For example, the mean E2020 plasma concentration in male rats one hour after a 3 mg/kg dose was 24.1 ng/ml on Day 1 of the one-year study and 87.4 ng/ml on Day 361. In male and female dogs, Cmax values in the 2.0 mg/kg/day group increased 3.07-fold from 19.48 on Day 1 to 59.80 ng/ml on Day 364, and the corresponding mean values for $AUC_{(0-\infty)}$ increased 5.80-fold from 69.71 to 404.3 ng·hr/ml.

3.1.2 Absorption and Pharmacokinetics of ^{14}C-labelled E2020: Single-dose Studies In rats, E2020 related radioactivity was rapidly absorbed, and displayed a mean peak radioactivity concentration 30 minutes after administration. Virtually complete oral absorption of E2020 in the fasted rat can be deduced from the high recovery of radioactivity in the urine plus bile (97.3 ± 1.0% of dose). In dogs, total radioactivity and intact E2020 reached peak concentrations 1.5 to 2 hours after oral administration.

3.1.3. Absorption and Pharmacokinetics of ^{14}C-labelled E2020: Multiple-dose Studies. Little accumulation of radioactivity occurred during 7 days of continuous oral administration of ^{14}C-E2020 to male rats. The ratio of the $AUC_{(0-10hr)}$ values for the seventh dose, relative to the first administration, was 122 ± 4.8 %. Comparable results were found in a second study, with $AUC_{(0-24hr)}$ values for the final administration on Day 14 about 37% higher than those for the first dose.

3.1.4 Protein Binding. Plasma protein binding of E2020-related radioactivity was studied *ex vivo* and the binding of E2020 was studied *in vitro*.

3.1.4.1 When studied *ex vivo* in samples collected 1.5 hours after dosing dogs and in samples collected 0.5 to 12 hours after dosing rats, mean protein binding of E2020-related radioactivity was found to be 58% in the dog and ranged from 58 to 64% in the rat.

3.1.4.2 When studied *in vitro* at concentrations from 28.0 to 579.9 ng/mL, the binding of E2020 to rat plasma protein ranged from 64.44 ± 3.56% to 73.63 ± 1.37%. In dog plasma, the protein binding of E2020 ranged from 81.91 ± 0.45 % to 86.90 ± 0.74 % when studied at concentrations from 26.8 to 565.9 ng/mL. In human plasma, the binding of E2020 was 88.38 ± 3.19 % at 17.0 ng/mL, 91.20 ± 1.06 % at 70.1 ng/mL, and 89.05 ± 1.16 % at 351.4 ng/mL.

3.1.4.3 The binding of E2020 to purified albumin and alpha-1-acid glycoprotein (alpha-1-AG) from rat, dog and human serum was also studied *in vitro*. In all three species, binding to albumin was higher than to alpha-1-AG. When the binding of E2020 to human, dog and rat albumin was studied at an added concentration of 1 micromolar (416 ng/mL), the percentages bound were 73.9 ± 0.4%, 44.0 ± 2.2% and 61.1 ± 0.7%, respectively.

3.1.4.4 Displacement studies were performed *in vitro* using purified human serum albumin, E2020, and the test drugs furosemide, digoxin and warfarin. The unbound percentage for E2020 was not affected by the presence of the test drugs, and the binding results for the test drugs were unaffected by E2020.

3.1.5. Tissue Distribution. Following single intravenous administration to male rats and dogs, the volumes of distribution (V_{dss}) for E2020 were large (≥ 10 L/kg), indicating extensive distribution into tissues.

3.1.5.1. Using the technique of whole-body autoradiography, 0.5 hours after administering a singe oral 1 mg/kg dose of ^{14}C-E2020 to fasted male rats, the highest concentrations of radioactivity were found in the liver, esophagus and bladder. Only low levels of radioactivity were found in the central nervous system with the pituitary gland and pineal body having slightly higher concentrations of radioactivity. By 168 hours after dosing, levels of radioactivity were too low for the distribution to be determined.

3.1.5.2. One-half hour after administering a single oral 1 mg/kg dose of ^{14}C-E2020 to fasted male rats, the highest concentrations of radioactivity were found in the gastrointestinal tract (administration site), liver, pancreas, pituitary, adrenals, kidneys and bone marrow. These were 48.7 to 11.4 times the corresponding plasma levels. The brain, liver and kidneys contained 0.19 ± 0.05, 14.0 ± 2.62 and 1.48 ± 0.34% of the dose, respectively, 0.5 hours after dosing. No radioactivity was detected in any tissue apart from the testis and the liver 168 hours after dosing, and the residues in these tissues corresponded to <0.01% of the administered dose in both cases.

3.1.5.3. In male dogs, relatively high levels (40.6 to 10.6 times the plasma concentrations) were found in the liver, ciliary body, kidney medulla, pancreas, choroidea and iris 1.5 hours after dosing. Virtually all of the other tissues also contained higher levels of radioactivity than the plasma. By 168 hours after dosing, the plasma level of radioactivity had decreased to 1.17% of maximum, and the liver contained a mean concentration which corresponded to 1.01 ± 0.10% of the administered dose. Mean concentrations in excess of 1000 ng-equiv./g were present in the ciliary body, choroidea and iris.

3.1.5.4. Although the majority of radioactivity was present as metabolites in rat and dog plasma shortly after the oral administration of ^{14}C-E2020, E2020 was the principal drug-related substance in the brains of both species.

3.1.6 Metabolism. The metabolism of E2020 was studied in rats and dogs in vivo, and in vitro using liver microsomal preparations from rats, dogs, and humans.

E2020 was extensively metabolized in the two main species used for toxicology studies. A total of 13 metabolites were isolated from rat urine and feces and two further compounds from an in vitro incubation of E2020 with dog liver microsomes. Of these products:

1. Six were primary phase 1 metabolites, formed by: 6-O-demethylation (M1), 5-O-demethylation (M2), para-hydroxylation of the benzene ring (M3), N-desalkylation (M4) and N-oxidation (equatorial and axial; M5 and M6, respectively).

2. Four were secondary phase 1 products (M7 - M10), which were formed by further oxidation of the primary metabolites.

3. Five were conjugates, formed either by glucuronidation of the 5- and 6-O-desmethyl products (M11 - M14), or by sulfation of the p-phenol (M7-sulfate).

The proposed metabolic routes for the formation of these metabolites are shown in the figure on the following page. In rat and dog plasma shortly after the oral administration of ^{14}C-E2020, the majority of radioactivity was present as metabolites. In rat plasma, O-glucuronides (55%) and E2020 (31% of the radioactivity) were the main drug related components. In dog plasma, 55% of the radioactivity was 0-glucuronides and 6.2% was E2020 In addition to these products, three unidentified metabolites (UM1 - UM3) were also present in rat and dog urine.

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=B,M,X;SUBSET=F

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	10	0	0	9
CERVICAL SC	NUMBER EXAMINED:	9	0	0	9
THORACIC SC	NUMBER EXAMINED:	10	0	0	9
LUMBAR SC	NUMBER EXAMINED:	10	0	0	9
NERVE	NUMBER EXAMINED:	10	0	0	9
HEART	NUMBER EXAMINED:	10	0	0	9
SALIVARY GLANDS	NUMBER EXAMINED:	10	0	0	9
AORTA	NUMBER EXAMINED:	10	0	0	9
TRACHEA	NUMBER EXAMINED:	10	0	0	9
ESOPHAGUS	NUMBER EXAMINED:	10	0	0	9
LUNGS	NUMBER EXAMINED:	10	6	5	9
MESENTERIC LN	NUMBER EXAMINED:	9	0	0	9
MEDIASTINAL LN	NUMBER EXAMINED:	10	0	1	9
THYMUS	NUMBER EXAMINED:	10	0	0	9
SPLEEN	NUMBER EXAMINED:	10	0	0	9
LIVER	NUMBER EXAMINED:	10	0	0	9
BONE STERNUM	NUMBER EXAMINED:	10	0	0	9
BONE FEMUR	NUMBER EXAMINED:	10	0	0	9
BONE RIB	NUMBER EXAMINED:	7	0	0	8
MARROW STERNUM	NUMBER EXAMINED:	10	0	0	9

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
	NUMBER:	10	10	10	9
MARROW FEMUR	NUMBER EXAMINED:	10	0	0	9
MARROW RIB	NUMBER EXAMINED:	7	0	0	8
STOMACH	NUMBER EXAMINED:	10	0	0	9
DUODENUM	NUMBER EXAMINED:	10	0	0	9
PANCREAS	NUMBER EXAMINED:	10	0	0	9
JEJUNUM	NUMBER EXAMINED:	10	0	0	9
ILEUM	NUMBER EXAMINED:	10	0	0	9
CECUM	NUMBER EXAMINED:	10	0	0	9
COLON	NUMBER EXAMINED:	10	0	0	9
RECTUM	NUMBER EXAMINED:	10	0	0	9
TONGUE	NUMBER EXAMINED:	8	0	0	7
KIDNEYS	NUMBER EXAMINED:	10	1	2	9
URINARY BLADDER	NUMBER EXAMINED:	9	0	0	8
TESTES	NUMBER EXAMINED:	10	0	0	9
EPIDIDYMIDES	NUMBER EXAMINED:	10	0	0	9
PROSTATE	NUMBER EXAMINED:	10	0	0	9
SEMINAL VESICLES	NUMBER EXAMINED:	10	1	0	9
COAGULATING GL	NUMBER EXAMINED:	10	0	0	9
PITUITARY GLAND	NUMBER EXAMINED:	9	0	0	9
THYROID GLAND	NUMBER EXAMINED:	9	0	0	9

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=B,M,X;SUBSET=1

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	9
PARATHYROID GLS.	NUMBER EXAMINED:	9	0	0	7
ADRENAL GLANDS	NUMBER EXAMINED:	10	0	0	9
EYES	NUMBER EXAMINED:	10	0	0	9
HARDERIAN GLANDS	NUMBER EXAMINED:	10	0	0	9
MUSCLE	NUMBER EXAMINED:	10	0	0	9
SKIN	NUMBER EXAMINED:	10	2	1	9
LYMPH NODE	NUMBER EXAMINED:	9	1	0	1
TAIL	NUMBER EXAMINED:	0	1	1	1

** END OF LIST **

T 25A-4
Table 25A (cont.)

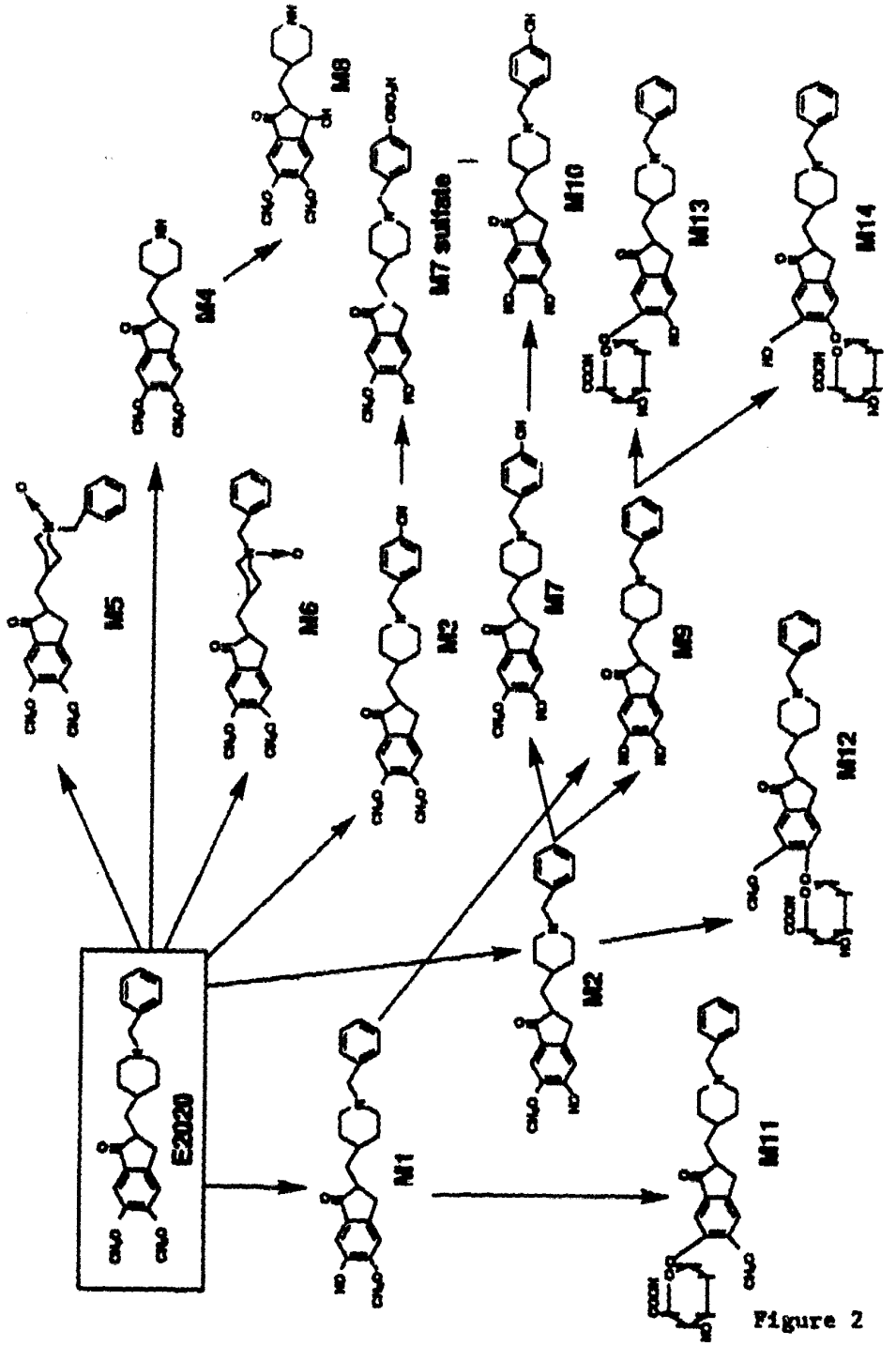


Figure 2

3.1.6.1. In male rats, the profile of metabolites was determined in the naturally excreted urine and feces that were collected during the first 24 hours after an oral dose of 1.0 mg/kg ^{14}C-E2020. In urine, the O-glucuronides (M11 - M14) were the main metabolites (mean ± SEM: 23.0 ± 2.57% of dose), followed by M4 (3.34 ± 0.20%), M1 (2.48 ± 0.89%) and E2020 (1.52 ± 0.23%). Other known metabolites were also present, but in amounts that averaged ≤ 1.01% of the dose.

In rat feces, M9 (13.5 ± 2.39%) was the main metabolite present, followed by O-glucuronides (6.87 ± 0.78%) and E2020 (4.21 ± 0.56%). Other metabolites that accounted on average for between 3 and 4% of the administered dose were M1, M4, M7-sulfate and UM1. The remaining metabolites were also present, but each accounted for ≤1.6% of the dose.

In dogs, the profiles of metabolites were analyzed using samples collected up to 48 hours. O-glucuronides were the main metabolites in urine (8.56 ± 0.87% of dose), followed by UM3 (2.45 ± 0.45%), M7-sulfate (2.07 ± 0.19%), M4 (1.56 ± 0.10%) and M2 (1.07 ± 0.19%). E2020 and the remaining metabolites were all present, but on average they each accounted for ≤0.4% of dose.

The largest single component in dog feces was the unidentified metabolite UM2 (13.6 ± 1.97% of dose). The main known metabolites in the feces were: M2 (12.2 ± 2.04%), the O-glucuronides (6.15 ± 1.14%) and M4 (6.04 ± 1.01%), but measurable quantities of all of the other identified compounds were also present.

3.1.6.2. *In Vitro* Studies. In human microsomes, M4 was the major metabolite formed, with smaller quantities of M1 and M2. These three products were also the main metabolites formed by male and female rat liver microsomes, together with smaller amounts of M3 in both genders, and low levels of M5 in the males. M2 and M1 were the main metabolites in male dog liver microsomes. In human microsomes, M4 was formed mainly by CYP3A4 (which is the major form of human cytochrome P450 and accounts for about 30%

of the total enzyme), while M1 and M2 are produced predominately by CYP2D6. Theophylline (a substrate for CYP1A1) did not inhibit the metabolism of E2020; cimetidine (a general inhibitor of cytochrome P450) showed a low affinity non-competitive inhibition of E2020 metabolism *in vitro*, and may result in only a limited interaction *in vivo*. Quinidine (a potent inhibitor of CYP2D6 and also a substrate for CYP3A4) weakly inhibited the formation of M1, M2 and M4 *in vitro*, and may have only a slight effect on the metabolism of E2020 *in vivo*. By contrast, erythromycin (a CYP3A4 substrate) competitively inhibited the metabolism of E2020 and might decrease its metabolism *in vivo*. Ketoconazole (a potent inhibitor of the CYP3A4) was a high affinity inhibitor of M1, M2 and M4 formation, and is expected to be a potent inhibitor of M4 formation *in vivo*. In a study of ^{14}C-labelled E2020 given to healthy volunteers (Protocol E2020-A001-004), substantial amounts of unchanged E2020, M1, M2, and their glucuronides were excreted. This indicates that E2020 is eliminated via multiple mechanisms. Thus, no single drug is likely to greatly increase plasma levels in humans.

3.1.7 Excretion. Following a single administration of ^{14}C-E2020 to dogs orally and to rats orally or intravenously, radioactivity was excreted rapidly in bile, urine, and feces. Virtually complete recovery of E2020-related radioactivity was achieved in rat and dog urine and feces; over 90% of the dose was recovered from both species within the first 48 hours after dosing.

3.1.7.1 In male rats, 91.2 ± 0.72% of the administered dose was recovered in the excreta in the first 24 hours after dosing, of which 36.9 ± 0.81% was in the urine and 54.3 ± 0.32% in feces, and over the full 168 hour study period the total recovery was 98.9 ± 0.77%, with 39.2 ± 0.65% and 59.7 ± 0.64% in the urine and feces, respectively.

3.1.7.2 In the dog, 74.3 ± 2.56% of the dose was excreted in the first 24 hours after administration, of which 17.8 ± 1.63% was recovered in urine and 56.5 ± 3.78% in the feces. The total recovery had increased to 90.4 ± 0.48% by 48 hours, after which the excretion was low. During the 168 hour period of the study, 98.3 ± 0.87% of

the dose was recovered, 21.4 ± 1.71% in the urine and 77.1 ±1.10 % in the feces.

3.1.8. Comparison with ADME Data from Humans. In the single-dose Phase I Study in the United States (Protocol E2020-A001-001), in which oral E2020 doses of 2.0, 4.0, and 6.0 mg were compared to placebo in healthy male volunteers, peak concentrations were achieved by approximately 4 hours after dosing, regardless of the E2020 dose. The relationship between AUC and dose was linear across the dose range tested. For the two highest doses tested (for which the assay provided complete characterization), the terminal half-lives were 82.8 ± 22.3 and 80.1 ± 21.6 hours, respectively, and the apparent volumes of distribution ($V_{\lambda z}/F$) were 12.8 ± 2.5 and 12.3 ± 1.9 L/kg, respectively. Thus, as might be expected, E2020 was cleared more slowly from man than from rats or dogs.

In another Phase I study in which a 5 mg dose of ^{14}C-E2020 (50 microcuries) was administered to healthy male volunteers, 57% of the dose was recovered from urine and 15% from feces over the ten days following dosing. This pattern of excretion is roughly the reverse of that observed in rats (39.2 ± 0.65% urine and 59.7 ± 0.64% feces) and dogs (17.8 ± 1.63% urine and 56.5 ± 3.78% feces). In human urine, parent E2020 comprised 16.9% of the recovered radioactivity, and the remaining metabolite pattern was as follows (M11 and M12 are O-glucuronidated versions of M1 and M2, respectively): M1=1.5%; M11=4.9%; M2=1.1%; M12=2.1%; M4=6.1%; M6=1.9%.

In human feces, parent E2020 accounted for 1% of the recovered radioactivity; of the identified metabolites, M1, M11, M2, M12, M4, and M6 were all present at less than 1.0% of the recovered dose.

Thus, it can be concluded that the metabolites in the two principal toxicology species adequately covered the metabolites in humans. There was no unique metabolite identified in humans that was not present in rats or dogs.

E2020 is eliminated via multiple pathways in humans, including metabolism by at least two different P450 isozymes, and excretion of unchanged E2020.

3.2 ABSORPTION AND PHARMACOKINETICS OF E2020; SINGLE DOSES

The absorption and single dose pharmacokinetics of E2020 were studied in Sprague-Dawley rats and Beagle dogs, the two main species used in toxicology.

An HPLC-UV assay was used to study the disposition of E2020 in rats and dogs in several studies. Following single intravenous administration to male rats and dogs, the elimination half-life in both species was about 3 hours. The volumes of distribution (V_{dss}) were large (≥ 10 L/kg), indicating extensive distribution into tissues and the plasma clearances were high (≥ 80 ml/min/kg) indicating a possible first-pass effect for oral administration. Following oral dosing, E2020 was rapidly absorbed by both species (t_{max} 0.5 to 2 hours). C_{max} and AUC values both showed a more than dose-proportional increase with increases in dose. Evidence that this was due to saturation of first-pass metabolism was provided by a study in rats, in which one group was dosed intravenously at 3.0 mg/kg and three other groups were dosed orally at 1.0, 3.0 and 10 mg/kg [D-1].

Table 1

Pharmacokinetic parameters of E2020 in plasma after oral and intravenous administration of single doses to rats.

Route	Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/ml)	$t_{1/2}$ (6-12hr) (hr)	$AUC_{(0-24hr)}$ (µg hr/ml)	CL_{total}* (ml/min/kg)	$Vd_{(ss)}$ (l/kg)	B.A. (%)
Intravenous	3.0	0.08 †	545 †	3.00	0.614	81.4	11.5	-
oral	1.0	0.50	43.3	6.30	0.119	140	38.2	58.1
oral	3.0	0.50	119	3.60	0.263	190	31.4	42.8
oral	10	0.50	638	4.98	1.84	90.6	20.4	89.9

*: Oral values are not corrected for bioavailability.
†: First sample collected, 5 min after administration.

(Source: Table 3 of D-1; PK-9; W-931117)

Comparing the mean Cmax and $AUC_{(0\text{-}24hr)}$ values with the corresponding value for the i.v. dose indicated absolute bioavailabilities of 58.1, 42.8, and 89.9 %, respectively. The higher bioavailability for the 10 mg/kg dose suggested that the 10 mg/kg dose tended to saturate the first-pass effect in the fasting rat. The elimination half-life for the i.v. dose was 3.00 hr, with corresponding values for the 1.0, 3.0 and 10 mg/kg oral doses of 6.30, 3.60 and 4.98 hours. A high Vd_{ss} of 11.5 L/kg for the i.v. dose suggested extensive tissue penetration.

When the same oral doses were administered to rats that were allowed continuous access to food [D-2], the non-linearity in the pharmacokinetics of E2020 was much less evident (Table 2). The 3.33 fold increment in the dose from 3.0 to 10 mg/kg produced a 3.66 and 4.14 fold increase in the mean Cmax and $AUC_{(0\text{-}8hr)}$ values, respectively, in male rats. Similar dose-proportionality was also apparent in the females. Thus, the oral pharmacokinetics of E2020 in non-fasting rats appeared to be linear.

Table 2: Pharmacokinetic parameters of E2020 in plasma following a single oral administration by gavage to non-fasted male and female rats.

Gender	Group Dose	T_{max} (hr)	C_{max} (ng/ml)	$AUC_{(0\text{-}8hr)}$ (ng·hr/ml)
Male	1.0 mg/kg	1.0 (0.25 - 1.0)	5.45 ± 2.63	9.04 ± 3.33
	3.0 mg/kg	0.5 (0.5 - 1.0)	19.6 ± 2.52	65.0 ± 7.61
	10 mg/kg	0.5 (0.5 - 0.5)	71.8 ± 2.90	269 ± 30.3
Female	1.0 mg/kg	1.0 (1.0 - 1.0)	14.7 ± 1.67	54.1 ± 6.03
	3.0 mg/kg	0.75 (0.5 - 1.0)	46.9 ± 7.50	200 ± 26.1
	10 mg/kg	0.75 (0.5 - 1.0)	152 ± 18.1	597 ± 46.3

Tmax values are median and range ; other parameters are mean ± SEM.

Although both genders absorbed the drug at comparable rates, the mean Cmax and $AUC_{(0\text{-}8hr)}$ for female rats were 2.12 to 5.98 times higher than those in males. This higher clearance in the male rat is consistent with the in vitro finding that the rate of metabolism by male rat liver microsomes is higher than the corresponding rate for the female rat (see below).

Mean Cmax values in non-fasting female rats were 2.1 to 2.7 times higher than those in males. This study is especially relevant to the gavage toxicology studies of E2020 in rats, because the animals in these studies had access to food *ad libitum*.

A single dose-proportionality and absolute bioavailability study was also performed in

fasted male dogs, using a cross-over design with an i.v. dose of 1.0 mg/kg in saline and oral doses of 1.0 and 3.0 mg/kg administered as solutions in gelatin capsules [D6]. The mean Cmax for the oral doses were 3.37 ± 0.58 and 49.5 ± 3.61 ng/ml, respectively, with $AUC_{(0-\infty)}$ values of 10.9 ± 1.55 and 159 ± 12.6 ng·hr/ml. Thus the 3-fold increment in the doses resulted in approximately a 14.6 fold increase in both parameters. Comparing the individual AUCs for the 1.0 and 3.0 mg/kg oral doses with their corresponding values for the i.v. administration (192 ± 11.2 ng·hr/ml) indicated absolute bioavailabilities of 5.85 ± 0.99 and 28.4 ± 3.0 %, respectively. This suggested that a saturable first-pass effect also occurs in fasting dogs. The mean elimination half-life for the i.v. dose was 3.04 ± 0.09 hr, and that for the 3.0 mg/kg oral dose was 3.28 ± 0.10 hours (a reliable value could not be determined for the 1.0 mg/kg dose, as plasma concentrations were only quantifiable up to 6 hours after dosing). Again a high Vd_{ss} of 17.4 ± 0.79 L/kg for the i.v. dose suggested extensive tissue penetration.

Single-Dose Studies of ^{14}C-E2020 in Rats and Dogs. Oral absorption of ^{14}C-labelled E2020 was investigated in fasted male rats [D-4] and dogs [D-7]. Absorption in both species was rapid, with peak blood concentrations of radioactivity being found 0.5 hours after dosing in rats, and 1.5 hours after dosing in dogs. Analysis of samples collected after an oral 1 mg/kg dose of ^{14}C-E2020 showed that E2020 accounted a for relatively small proportion of the drug related material in plasma (35% of Cmax in the rat and 6.21% in the dog) [D-4, D-7]; this suggested that extreme metabolism was occurring as part of a "first-pass" effect. Nevertheless, virtually complete oral absorption of E2020 in the fasted rat can be deduced from the high recovery of radioactivity in the urine plus bile (97.3 ± 1.0% of dose, described further below).

The concentration - time curve for total radioactivity in the rat showed a number of secondary maxima, suggesting biliary elimination and entero-hepatic circulation (EHC). This conclusion was supported by administering ^{14}C-E2020 intravenously to rats [D-5] in a second study which showed a secondary peak in the radioactivity profile 4 hours after dosing. It was confirmed by administering radiolabelled compound, both orally and intravenously, to rats with a cannulated bile-duct [D-4, D-5]; the secondary maxima were no longer observed in the plasma profiles of the cannulated rats. In addition, the plasma AUC values for total radioactivity were reduced in the cannulated animals by about 50% (after oral administration) and 30% (after intravenous administration), compared to the intact rats. However, these secondary maxima were not seen in the profiles for unchanged drug in rat, suggesting that the EHC involved metabolites rather than E2020 itself [D-1, D-2, D-4]. Moreover they were not seen in the total radioactivity profiles in the dog [D-5], suggesting that the phenomenon was specific to the rat.

3.3 MULTIPLE DOSE PHARMACOKINETICS/TOXICOKINETICS

Multiple dose pharmacokinetics of E2020 were studied in male and female rats, mice, and dogs. A more complete profile was obtained in the rat than in the mouse, so the information on the rat will be presented first.

Multiple-dose Rats. Five repeated dose studies were performed in the rat [D-8 to D-12].

1. To assesses the accumulation of E2020 under the conditions used for toxicity studies, drug levels were measured in plasma of rats (n = 4 males and females/group) that were dosed orally by gavage for 15 days, at 1.0, 3.0 and 10 mg/kg while they had continuous access to food and water [Table 3; D-8].

Table 3: Mean pharmacokinetic parameters of E2020 in plasma (± SEM) during repeated gavage administration to non-fasting male and female rats.

Gender	Dose	C_{max} (ng/ml)		T_{max} (hr)		$AUC_{(0\text{-}8hr)}$ (ng·hr/ml)	
	mg/kg	1st day	15th day	1st day	15th day	1st day	15th day
Male	1.0	8.43 ± 4.78	5.99 ± 1.07	0.5	2.0	22.8 ± 6.53	25.1 ± 4.85
	3.0	18.7 ± 3.18	18.6 ± 2.02	1.25	2.0	73.9 ± 12.0	79.0 ± 9.35
	10.0	99.3 ± 3.53	91.2 ± 13.1	0.5	2.0	291 ± 22.4	476 ± 81.3
Female	1.0	16.9 ± 2.79	13.2 ± 0.71	1.25	2.0	60.7 ± 4.22	63.2 ± 3.14
	3.0	67.8 ± 9.60	68.9 ± 5.52	0.5	2.0	270 ± 27.3	337 ± 38.7
	10.0	147 ± 16.0	291 ± 109	0.5	0.5	705 ± 64.2	1225 ± 205

Mean ± SEM are calculated for four animals, except for Tmax which is summarized as the Median.

The Cmax and $AUC_{(0\text{-}8hr)}$ on both study days were higher in females than males, thus confirming the gender difference seen in the single dose study. Comparing the Cmax and AUC on the first and final (15th) days showed no evidence of accumulation of E2020, nor a change in its pharmacokinetics, for either sex dosed at 1.0 mg/kg, or the males at 3.0 mg/kg/day. However E2020 accumulation was apparent in the females at the 3.0 and 10 mg/kg doses, as the AUC values for day 15 were significantly greater than those on the first day. Moreover, the accumulation probably exceeded that expected for first order kinetics, as the mean $AUC_{(0\text{-}24hr)}$ for each of the high dose females on Day 15 (2065 ± 168 ng·hr/ml) was greater than the corresponding $AUC_{(0\text{-}\infty)}$ for the first dose (1528 ± 206 ng·hr/ml; p=0.051).

2. Exposure to E2020 during the one year gavage toxicity study was assessed by analyzing pooled plasma samples collected from groups of satellite animals during weeks 1, 5, 27 and 52 [Table 4; D-9].

Table 4: Concentrations of E2020 in plasma at 1 hour after administration by gavage to male (M) and female (F) rats during a 1 year toxicity study.

		Dosage Group					
Study week	Day	1.0 mg/kg/day		3.0 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F
1	1	7.42	10.02	24.10	57.10	61.73	85.68
	3	13.98	11.19	32.33	55.32	92.09	104.22
5	1	6.85	17.33	40.99	75.00	157.07	159.81
	3	10.39	16.21	41.34	71.30	187.53	236.38
27	1	13.71	22.03	54.45	104.00	189.24	239.45
	3	10.58	18.82	47.17	63.93	149.50	210.33
52	1	12.95	23.95	65.34	108.42	224.06	304.64
	3	19.87	25.92	87.39	100.75	233.66	241.97

The results showed that, consistent with the other studies in which E2020 was administered to both genders, plasma concentrations were generally higher in the female rats. Moreover, the plasma levels of E2020 tended to increase during the study. For example, the samples collected from the males at 1 hour after administration on Day 1 contained mean E2020 concentrations of 7.42, 24.10 and 61.73 ng/ml, respectively, for the 1, 3 and 10 mg/kg dose groups, whereas the corresponding levels during the final week were 12.95, 65.34 and 224.06 ng/ml.

3. Exposure to E2020 during a 13 week dietary oral toxicity study, in rats, with target doses of 0, 15, 30 and 60 mg/kg/day, was assessed [Table 5; D-10]. On Day 15, the highest mean concentrations were found between 00.00 and 06.00 hours, and the minimum levels generally occurred at 15.00 hr. Such patterns are consistent with the normal (nocturnal) eating habits of rats.

Table 5: Pharmacokinetic parameters of E2020 in rat plasma on Day 15 of a 13 week dietary administration toxicity study.

Dose	C_{max} (ng/ml)		C_{min} (ng/ml)		$AUC_{(0\text{-}24hr)}$ (ng·hr/ml)	
(mg/kg)	male	female	male	female	male	female
15	105	242	27.4	67.0	1713	3215
30	238	574	48.3	118.0	3180	7663
60	810	1379	168.4	402.9	10170	19939

Consistent with results for gavage administration, the average exposure to E2020 in female rats was approximately twice as high as that in males. Even though the doses were higher, the dietary toxicokinetics of E2020 in rats were essentially linear. Thus, the 4-fold increment in target doses (which, in practice, because of differences in the amounts of food consumed was closer 4.5 fold) resulted in approximately a 6-fold increase in AUC values for both the male and female rats. Also, the comparison of the concentrations at 03.00 hr on Day 85 with the corresponding Day 15 values showed that the exposure to E2020 effectively remained constant during the study (Table 14, narrative for Study Reference D-10).

4. As single dose studies showed that total radioactivity was cleared more slowly than E2020, 1.14 mg/kg ^{14}C-E2020 was administered orally to male rats for 7 days, assess the accumulation of drug related material [D-11]. After both the first and final doses, the concentration-time profiles for blood radioactivity showed the secondary maxima seen with single doses of ^{14}C-E2020 in rats. However, the extent of accumulation was limited, as the ratio of the $AUC_{(0\text{-}10hr)}$ values for the seventh dose, relative to the first administration, was only 122 ± 4.8 %.

5. Comparable results were found in a second multiple-dose 14C-E2020 study, in which 1.0 mg/kg doses were administered by gavage to non-fasted male rats for 14 days [D-12]. $AUC_{(0\text{-}24hr)}$ for the final administration (mean ± SD: 1081 ± 35 ng-eq.·hr/ml) were slightly but significantly higher than those for the first dose (786.2 ± 64.3 ng-eq.·hr/ml), indicating that the exposure to E2020 and/or its metabolites increased by ca. 38% during the 14 days of dosing.

Multiple-dose Mice. The toxicokinetics of E2020 were studied in mice, as part of a 13 week toxicity trial with dietary administration, at target doses of 0, 15, 30, 60 and 90 mg/kg/day [D-13]. Using satellite animals, the plasma concentration-time profiles of E2020 were measured on Day 15 (Table 6).

Table 6: Pharmacokinetic parameters of E2020 in mouse plasma, on Day 15 of a 13 week dietary administration toxicity study.

Dose (mg/kg)	C_{max} (ng/ml) male	C_{max} (ng/ml) female	$AUC_{(0\text{-}21hr)}$ (ng·hr/ml) male	$AUC_{(0\text{-}21hr)}$ (ng·hr/ml) female
15	11.8*	13.4*	129.21	125.34
30	34.1	35.5	441.56	346.38
60	195.0	114.7	2310.44	1337.33
90	311.0	207.7	4622.55	3120.14

*BQL: below the assay LOQ of 25 ng/ml

The highest concentrations were generally found at midnight (00.00 hr) with minimum levels between 12.00 and 18.00 hr. Such patterns are consistent with the nocturnal eating habits of rodents. The target dose of 15 mg/kg produced comparable systemic exposures in male and female mice ($AUC_{(0\text{-}21hr)}$: 129 and 125 ng·hr/ml) but at the higher doses the levels were generally higher in males than females. Moreover, the increase in exposure with dose was more than proportional for each group, especially at the higher doses; for example the two fold increment from 30 to 60 mg/kg/day produced about a 4-fold increase in the AUC values in female animals (346 and 1337 ng·hr/ml) and more than a 5-fold increase in the males (442 to 2310 ng·hr/ml). Again, this effect could be due to the partial saturation of a first-pass effect when higher doses are administered.

Determining the concentrations at 00.00 hr on Day 85, for comparison with the corresponding values on Day 15, indicated that the exposure to E2020 remained constant throughout the remainder of the study (see Table 16, narrative for Study Reference D-13).

Multiple-dose Dogs. Exposure to E2020 during the 13 week [D-14] and 1 year toxicity trials [D-15] was assessed by direct toxicokinetic monitoring of 3 male plus 3 female dogs in each dosage group. E2020 was administered by the method used in the toxicity trials, i.e., administration once-a-day of E2020 powder mixed with lactose and loosely packed in a capsule. In evaluating the results, because there was no clear evidence of gender difference in these studies, the results for both sexes were pooled.

The target doses for the 13 week study were 0.3, 1.0, 3.0 and 8.0 mg/kg/day; however, unexpected mortality at the top dose during the first two days necessitated a revision of the experimental design, and the majority of survivors in the top dose group were subsequently dosed at 3.0 mg/kg/day. The assay method was sufficiently sensitive to monitor the plasma levels of E2020 for the 1.0 and 3.0 mg/kg doses, and the concentration-time profiles in these groups were determined on Days 1, 35 and 91, with

additional monitoring of the Cmin values on the 4th and 8th days.

In the animals dosed at 1.0 mg/kg, all of the Cmin were BQL. However, the values for the 3.0 mg/kg group indicated that, while an initial accumulation of E2020 occurred during repeated administration, a steady state was established by Day 35 (Table 7).

On Day 1 of the study, the $AUC_{(0\text{-}8hr)}$ values for the 1.0 and 3.0 mg/kg doses were 12 ± 5 and 140 ± 35 ng·hr/ml, respectively. Both values were higher by Day 35 (20 ± 6 and 598 ± 107 ng·hr/ml, respectively) but were then essentially unchanged on Day 91 (27 ± 9 and 550 ± 108 ng·hr/ml).

Table 7 Mean pharmacokinetic parameters of E2020 in dog plasma (± SEM) during a 13 week oral toxicity study with powdered drug.

	Dose: 1.0 mg/kg/day			Dose: 3.0 mg/kg/day		
Study Day	Cmax (ng/ml)	Cmin (ng/ml)	AUC(0-8hr) (ng·hr/ml)	Cmax (ng/ml)	Cmin (ng/ml)	AUC(0-8hr) (ng·hr/ml)
1st	4.0 ± 1.4	NS	12 ± 5	54 ± 12	ND*	140 ± 35
35th	6.6 ± 2.0	BQL	20 ± 6	154 ± 18	8.3 ± 4.3	598 ± 107
91st	8.9 ± 2.6	BQL	27 ± 9	153 ± 26	8.6 ± 3.7	550 ± 108

NS: sample not collected at 24 hr after the first administration
BQL: < 1.0 ng/ml for analysis of 1 ml sample (* < 2.0 ng/ml for analysis of 0.5 ml sample)

Comparable results were also found in the one year study, in which the dogs were dosed at 0.6, 2.0 and 5.0 mg/kg/day, and concentration-time profiles were determined on Days 1, 28, 182 and 364.

For all three groups, the mean $AUC_{(0\text{-}24hr)}$ ± SD values for Day 1 (11.79 ± 4.10, 69.71 ± 39.88 and 538.3 ± 290.0 ng·hr/ml) were significantly lower than those on Day 28 (20.62 ± 11.19, 248.7 ± 102.4 and 1927 ± 532.4 ng·hr/ml), and while there was a trend to higher levels during the second six months, the change was (for example, $AUC_{(0\text{-}24hr)}$ values on Day 364 were: 39.54 ± 17.02, 404.3 ± 82.06 and 3336 ± 1194 ng·hr/ml).

3.4 PROTEIN BINDING

Ultracentrifugation of plasma samples collected from rats up to 12 hours after a single oral 1 mg/kg dose of ^{14}C-E2020 showed that the protein binding of radioactivity averaged <65% [D-4]. As E2020 accounted for 0 - 33% of the drug related material in these samples, its metabolites are not highly bound to rat plasma protein. In the companion dog study [D-7], the binding at 1.5 hr was 57.5 ± 1.03%. Since the intact drug accounts for 6.21% of the radioactivity at this time after a 1 mg/kg dose, E2020 metabolites are also only weakly bound by dog plasma proteins.

When studied *in vitro* at concentrations from 28.0 to 579.9 ng/mL, the binding of E2020 to rat plasma protein ranged from 64.44 ± 3.56% to 73.63 ± 1.37%. In dog plasma, the protein binding of E2020 ranged from 81.91 ± 0.45 % to 86.90 ± 0.74 % when studied at concentrations from 26.8 to 565.9 ng/mL. In human plasma, the binding of E2020 was 88.38 ± 3.19 % at 17.0 ng/mL, 91.20 ± 1.06 % at 70.1 ng/mL, and 89.05 ± 1.16 % at 351.4 ng/mL.

The binding of E2020 at concentrations of 0.5 to 1000 μM, to purified albumin and alpha-1-acid glycoprotein (alpha-1-AG) prepared from rat, dog and human serum proteins, was also studied by equilibrium dialysis [D-17]. In all three species binding to albumin was higher than to alpha-1-AG, suggesting that albumin is the more predominant binding protein. Also, for a given concentration, E2020 was more highly bound to human than dog and rat albumin. For example, at an added concentration of 1 micromolar (416 ng/mL), the percentages bound were 73.9 ± 0.4%, 44.0 ± 2.2% and 61.1 ± 0.7%, respectively.

To investigate the protein binding interactions between E2020 and other drugs, displacement studies were performed with three marketed products (furosemide, digoxin and warfarin), using equilibrium dialysis or ultrafiltration and purified human serum albumin [D-18]. Within the concentration range of 0.3 to 10 microgram/ml, the unbound percentage for E2020 was 20.8 to 28.3, and was not affected by the presence of the test drugs. Similarly, the results for the other drugs showed that, in the absence of E2020, the unbound percentage of furosemide was 0.9 ± 0.0%, and this was not changed by adding E2020 concentrations of 0.3 to 10 microgram/ml (which exceeds the C_{max} for clinical doses of E2020). Likewise, the percentages of unbound ^{3}H-digoxin and ^{14}C-warfarin (73.5 ± 0.7% and 0.6 ± 0.0%, respectively in the absence of E2020) were also unaffected by the presence of E2020.

3.5 TISSUE DISTRIBUTION

The distribution of radioactivity after single doses of ^{14}C-E2020 was studied qualitatively in male Sprague-Dawley rats by whole body autoradiography [D-3], and quantitatively in male Sprague-Dawley rats [D-4] and Beagle dogs [D-7] by LSC of tissue digests. In both species even at the first sampling time (0.5 hr in the rat and 1.5 hr in the dog, which are their respective Tmax for plasma radioactivity), the eliminating organs (liver, gastrointestinal tract, kidneys and gall-bladder in the dog) and associated body fluids (bile and urine) tended to contain the highest concentrations of radioactivity. However virtually all of the other tissues also contained higher levels of radioactivity than the plasma, indicating a rapid and extensive uptake of drug related material into the tissues of both species. The concentrations of radioactivity in the brain were about 2 times and 1.4 times the plasma levels in the rat and dog, respectively, and were similar in all regions.

Subsequently, the concentrations in most of the tissues declined at comparable rates to the levels in plasma. In rat plasma the concentrations were non-quantifiable (<1.35 ng-equiv./ml) 48 hours after dosing, but measurable levels (2.58 ± 0.33 ng-equiv./ml) were still present in dog plasma 168 hours after dosing. In the rat by this time only the liver and testis contained quantifiable levels of radioactivity (both of which accounted for at most 0.01% of the dose), but high concentrations were still present in certain dog tissues. In particular the livers still contained 1.01 ± 0.10% of the administered radioactivity, and mean concentrations in excess of 1000 ng-equiv./g were present in the ciliary body, choroidea and iris.

The contributions of drug and metabolites to the primary pharmacological action of E2020 was assessed by analysis of rat and dog brain samples collected in the quantitative distribution studies with ^{14}C-E2020. HPLC analysis of the rat brains collected from 0.5 to 8 hours after dosing showed that E2020 accounted for more than 85% of the drug related material [D-4]. Similarly TLC profiling indicated that 81 ± 4% of the radioactivity in dog brain at 1.5 hr after dosing was present as intact drug [D-7].

The uptake and elimination of E2020 by rat brain was investigated in the dose proportionality and absolute bioavailability study, in which a single i.v. dose (3.0 mg/kg) and three oral doses (1.0, 3.0 and 10 mg/kg) were administered to parallel groups of male rats [D-1]. The mean brain concentration at 5 min after intravenous administration (5790 ± 109 ng/g) was ca. 10 times higher than the plasma level (545 ± 28.8 ng/ml), demonstrating rapid and extensive penetration into the CNS. Moreover the elimination

(t1/2 2.67 hr) paralleled that in plasma, and the AUC(0-24hr) of 4730 ng·hr/g was ca. 8 times higher than the plasma value (614 ng·hr/ml).

E2020 was also rapidly and extensively taken up by the CNS of the male rat after oral administration. Thus Tmax for all three doses was 0.5 hr, and the AUC(0-24hr) (980, 2130 and 13300 ng·hr/g, respectively) were 7.23 to 8.24 times the plasma values (119, 263 and 1840 ng·hr/ml). As was th case for the plasma, exposure of the brain to E2020 was reasonably dose proportional at 1.0 and 3.0 mg/kg, but the increase at 10 mg/kg was disproportionately high. However the half-lives (6.60, 3.49 and 4.78 hr) were similar for all three doses, and were virtually identical to the plasma values (6.30, 3.60 and 4.98 hr). This supported the conclusion that any non-linearity at the highest dose was due to saturation of a first-pass effect, which in turn was supported by measuring the E2020 concentrations in liver samples collected from the same rats.

At 5 min after i.v. administration, the mean concentration of E2020 in liver was 8590 ± 701 ng/ml. This was followed by a biphasic decline with a terminal $t_{1/2}$ of 5.01 hr, and the AUC(0-24hr) was 26300 ng·hr/g. Tmax for all three oral doses was 0.25 hr, rather than the 0.5 hr found for plasma and brain; however the elimination half-lives (5.59, 3.69 and 5.44 hr, for the 1.0, 3.0 and 10 mg/kg doses) were comparable to the values for plasma and brain. Moreover the C_{max} values (7080, 19400 and 69800 ng/g) were 215 to 462 times higher than the corresponding plasma levels and were essentially dose proportional. Likewise, the $AUC_{(0\text{-}24hr)}$ (15400, 29100 and 100000 ng·hr/g) were linearly related to dose, but were only 129, 111 and 54.3 times higher than the levels in plasma. Thus the liver levels did not directly parallel the systemic concentrations during the absorption and distribution phases, but the terminal elimination rates were similar to those of both plasma and brain.

3.6 METABOLISM

E2020 was extensively metabolized in the two main species used for toxicology studies. A total of 13 metabolites that were isolated from rat urine and feces were characterized by MS and NMR spectroscopy [D-19], and two further compounds (stereo-isomeric forms of the same metabolite) were characterized after an in vitro incubation of E2020 with dog liver microsomes [D-20]. Of these products:

1. Six were primary phase 1 metabolites, formed by: 6-O-demethylation (M1), 5-O-demethylation (M2), para-hydroxylation of the benzene ring (M3), N-desalyklation (M4) and equatorial and axial N-oxidation (M5 and M6, respectively). The structures of all of these compounds were confirmed by independent chemical synthesis.

2. Four were secondary phase 1 products (M7 - M10), which were formed by further oxidation of the primary metabolites.

3. Five were conjugates, formed either by glucuronidation of the 5- and 6-O-desmethyl products (M11 - M14), or by sulfation of the p-phenol (M7-sulfate). One of the glucuronide conjugates was synthesised biochemically, by incubating M1 with UDP-glucuronic acid and dog liver microsomes.

The proposed metabolic routes for the formation of these metabolites are shown in Figure 2 above (Section 5.D.3.1.6). In addition to these products, three unidentified metabolites (UM1 - UM3) were also present in rat urine.

The relative amounts of these metabolites were investigated in rat plasma and four tissues collected at 0.5 hours after oral administration of ^{14}C-E2020 [D-4]. In the plasma, O-glucuronides (55%of the radioactivity) and E2020 (31% of the radioactivity) were the main drug related components. By contrast, E2020 was the major component in the brain (90%), pancreas (96%), liver (81%) and kidney (72%), indicating that the metabolites were largely confined to the general circulation. The main metabolites found in the liver and kidney were M4 (4.7% and 8.1 % of the radioactivity) and the O-glucuronides (4.2% and 15%, respectively).

A similar profiling of drug and metabolites was performed with three dog tissues and the bile recovered from the gall-bladder at 1.5 hours after an oral dose of ^{14}C-E2020 [D-7]. E2020 was again the main radiolabelled component in the brain (81%), pancreas (79%), liver (27%) and kidney (39%). As in the rat, however, the liver and kidneys contained relatively high proportions of M4 (7% and 16%) and the glucuronides (22% and 17%, respectively), and these were major components in both the plasma (M4 8.3% and O-glucuronides 55%, with 6.2% as E2020) and the bile (M4 and conjugates both accounted for 32% of the radioactivity with 1.5 % as E2020).

Additional metabolic studies were also performed in vitro, with rat, dog and human liver microsomes. A study with human microsomes showed that M1, M2 and M4 were likely to be the main metabolites of E2020 in man, and no unidentified products were formed [D-21]. These three products were also the main metabolites formed by male and female rat liver microsomes, together with smaller amounts of M3 in both genders, and low levels of M5 in the males. However, consistent with the lower plasma levels of E2020 in male rats *in vivo* [D-10 - D-12], the drug was metabolized far more rapidly by male than female rat liver microsomes (total Cl_{int} 33.7 versus 12.6 microliter/min/kg) [D-21]. When incubated with male dog liver microsomes, M2 and M1 were the main metabolites

(Cl_{int} 24.3 and 10.3 microliter/min/kg) together with smaller quantities of M4 (Cl_{int} 1.63 microliter/min/kg), M5 (Cl_{int} 0.74 microliter/min/kg) and M3, giving a total clearance (37.0 microliter/min/kg) which was intermediate between that in the male and female rat. By contrast M4 was the major metabolite formed in human liver microsomes (Cl_{int} 5.09 microliter/min/kg), with smaller quantities of M1 and M2 (Cl_{int} 0.71 and 0.55 microliter/min/kg, respectively). Moreover, these results indicate that the capacity of human liver to metabolize E2020 (total Cl_{int} 6.35 microliter/min/kg) is much lower than that of the rat or dog [D-21]. A study to identify which human P450 isoenzymes were involved in the formation of these metabolites indicated that M4 was formed mainly by CYP3A4 (which is the major form of human cytochrome P450 and accounts for about 30% of the total enzyme), while M1 and M2 are produced predominately by CYP2D6 [D-22]. Although the latter isoenzyme is subject to genetic polymorphism, this should not unduly influence the clearance of E2020 *in vivo* as this form should play only a minor role in the metabolism in man.

Finally, three studies assessed the potential for metabolic interaction with other drugs [D-23, D-24, D-25]. In the first study, IC_{50} values for E2020 were determined in human liver microsomes *in vitro* against isoform-selective substrates of cytochrome P450 1A2, 2C9, 2C19, 2D6, and 3A4 and were all greater than 100 micromolar. Ki values for the interaction of E2020 with CYP3A4 and CYP2D6 were also determined. Mean Ki values for three individual sets of human liver microsomes were 131 and 47 micromolar for CYP3A4 and CYP2D6, respectively. Both interactions were typical of competitive inhibition. Since E2020 anticipated therapeutic concentrations (ca. 150 nM) are more than 300-fold lower than the lowest Ki value obtained with CYP2D6 and almost 900-fold lower than the Ki observed with CYP3A4, E2020 is not expected to interfere with the metabolism of other drugs. As E2020 is predominantly metabolized by CYP3A4, metabolic interactions, if they occur, are most likely to occur with other drugs that are substrates or inhibitors of this isoenzyme. Consistent with this prediction, theophylline (a substrate for CYP1A1) did not inhibit the metabolism of E2020. Cimetidine (a general inhibitor of cytochrome P450) showed a low affinity non-competitive inhibition of E2020 metabolism *in vitro*. Likewise, quinidine (a potent inhibitor of CYP2D6 and also a substrate for CYP3A4) weakly inhibited the formation of M1, M2 and M4 *in vitro*. By contrast, erythromycin (a CYP3A4 substrate) competitively inhibited the metabolism of E2020, while ketoconazole (a potent inhibitor of CYP3A4) was a high affinity inhibitor of M1, M2 and M4 formation, and is expected to be a potent inhibitor of M4 formulation by CYP3A4 *in vivo*. The results of these studies also confirmed that M1 and M2 are mainly formed by CYP2D6, and to a lesser extent by CYP3A4, while the

formation of M4 is predominately mediated by CYP3A4 with little contribution from the other P450 isoenzymes. In humans, unchanged E2020, M4, M1 (and its glucuronide) and M2 (and its glucuronide) represent 18, 6.6, 7.3, and 3.9% of the recovered radioactivity that is excreted in the 10-days following a single 5 mg dose of 14C-E2020 to healthy volunteers. This indicates that E2020 is eliminated via urinary excretion of unchanged E2020 as well as metabolism by CYP3A4 and CYP2D6. Thus, no one drug is likely to block all routes of elimination.

3.7 EXCRETION

Excretion-balance studies in the male rat were performed with ^{14}C-E2020 (1.0 mg/kg), given as a single oral dose, repeated oral administration for 7 days and a single i.v administration. Relatively rapid excretion with high recoveries were found in each case. Moreover, the rates of excretion and the proportions of the doses that were recovered in the urine and feces were essentially independent of the route and/or the number of administrations. These findings are illustrated by the mean ± SEM recoveries from 0 to 24 hour and during the complete collection periods, which are listed below as percent of the administered dose (Table 8).

Table 8

Mode of	Urine		Feces		Urine + Feces	
Administration	24 hr	full	24 hr	full	24 hr	full
Single oral dose	36.9 ± 0.8	39.2 ± 0.7	54.3 ± 0.3	59.7 ± 0.6	91.2 ± 0.7	98.9 ± 0.8
Repeat oral dose 1	37.3 ± 3.0		58.7 ± 6.7		96.0 ± 5.1	
Repeat oral dose 7	37.6 ± 0.6	38.0 ± 0.5	59.9 ± 0.8	60.0 ± 0.8	97.5 ± 0.7	98.0 ± 0.7
Single i.v. dose	38.8 ± 2.3	40.7 ± 2.4	55.8 ± 2.7	62.1 ± 1.3	94.6 ± 0.9	102.8 ± 1.2

TLC profiling of the 0 to 24 hour collections from the single p.o. dose showed that the O-glucuronides (M11-M14) accounted for the majority of the radioactivity in urine (23.0% of the dose), together with relatively high amounts of M4 (3.3%), M1 (2.5%) and E2020 (1.5%). The remaining metabolites were also excreted but in quantities ≤ 1.0% of the dose. E2020 and its characterized metabolites accounted for 34.2% of dose; i.e. virtually all of the radioactivity in this urine sample. In the feces M9 (13.5%) was mainly present, followed by O-glucuronides (6.9%) and E2020 (4.2%). M1, M4, M7-sulfate and UM1 accounted for ` to 4% of the dose, and the remaining metabolites were also present, but each accounted for ≤ 1.6% of the dose. In the 0 to 24 hour feces, E2020 and its characterized metabolites accounted for 42.2% of the dose. The fact that O-glucuronides (conjugates of M1 and M2) were the major metabolites in vivo agreed with

the results obtained with rat liver microsomes [D-19 and D-21].

Excretion-balance in male dogs showed that a smaller proportion of the dose (74.3 ± 2.6%) was excreted in the first 24 hr after dosing, of which 17.8 ± 1.63% was recovered in urine and 56.5 ± 3.78% in feces. However the recovery after 48 hr had increased to 90.4 ± 0.48%, and during the 168 hr study period a total of 98.3 ± 0.87% of dose was recovered, 21.4 ± 1.71% in the urine and 77.1 ±1.10 % in the feces.

The profiles of metabolites were analyzed using the samples collected to 48 hours. O-glucuronides were the main metabolites in urine (8.6% of dose), followed by UM3 (2.5%), M7-sulfate (2.1%), M4 (1.5%) and M1 (1.1%). E2020 and the remaining metabolites were all present, but they each averaged ≤0.4% of dose. The urine sample analyzed contained 19.7% of the dose, of which 14.9% was identified as E2020 and known metabolites. The largest component in feces was UM2 (13.6% of the dose), and the main known metabolites were M2 (12.2%), the O-glucuronides (6.2%) and M4 (6.0%), but measurable quantities of all of the other identified compounds were also present. Overall in the feces, 42.0% of the dose corresponded to E2020 and its known metabolites, compared with a total of 70.6% of dose in the sample analyzed. The high recoveries of the O-desmethyl metabolites (M1, M2 and their glucuronides) were again consistent with the in vitro metabolism results with dog liver microsomes [D-21].

The high fecal recoveries of radioactivity after i.v. administration to the rat, as well as following oral doses in both the rat and dog, suggested extensive biliary elimination of drug related material. This was confirmed directly, by measuring the biliary excretion after both a p.o. and i.v. dose in the rat. Bile samples collected up to 24 hr after the i.v. dose contained 76.9% of dose, of which <0.7% was intact E2020. Within 2 hr of the oral dose, on average 48.6% of the administered radioactivity was recovered in bile. By 6, 12, 24 and 48 hr after dosing this had increased to a mean of 64.2%, 70.1%, 72.2% and 72.9%, respectively. The corresponding 48 hour excretions in urine and feces were 24.4 % and 8.8 %, giving a total recovery of 106 ± 1.9% of the dose. Virtually complete oral absorption of E2020 in the fasted rat could also be deduced from the high recovery of radioactivity in the urine plus bile (97.3 ± 1.0% of dose).

Full metabolite profiles were determined in the 0 - 24 hr collections after the oral dose. On average, E2020 accounted for 0.22% of the excreted radioactivity. O-glucuronides were the major components (65.7% of radioactivity), followed by UM3 (8.4%), M4 (6.8%) and M7-sulfate (2.1%). This confirms that E2020 can only undergo very little entero-hepatic recirculation; therefore, the secondary maxima in the blood concentration-time profiles for total radioactivity (seen in intact but not bile duct cannulated rats; [D-4, D-5]) must be due to EHC of metabolites (probably the aglycones of the glucuronide

conjugates) rather than parent drug.

3.8 COMPARISON WITH ADME DATA FROM HUMANS

A comparison between animal and human ADME results is included in Section 5.D.3.1.8 of the Overview above.

3.9 BIOANALYTICAL ASSAY METHODS

3.9.1. HPLC ASSAY OF UNLABELLED E2020

The same basic procedure was used for all of the studies to quantify E2020. After adding an internal standard (IS), the pH was adjusted with alkali, and E2020 plus IS were extracted into organic solvent. The test compounds were then extracted with a small volume of acid, and an aliquot was analyzed by HPLC-UV. Peak height ratios for E2020/IS were fitted to the equation $Y = A \cdot X + B$, by a weighted regression.

The first method developed, for analysis of rat plasma, was evaluated for intra-assay variability [D-1]. A linear response with acceptable accuracies (- 6.6% to + 9.5%) was found at concentrations of 2.00 to 1000 ng/ml. Stability during storage was also assessed using Quality Control Standards (QC's) prepared in plasma at 10 and 500 ng/ml; after 3 months at -80°C, the mean found concentrations were within 10% of the nominal concentrations. The procedure was also evaluated for the quantification of E2020 in homogenized samples of rat brain, at concentrations of 2.00 to 2000 ng/2 ml (intra-assay accuracies: -11.4 to + 6.0%) and liver at 5.00 to 10,000 ng/ml (-12.3 to + 6.0%).

A similar procedure [D-14] was used to analyze samples of dog plasma collected during the 13 week toxicity study. Assay performance during analysis was assessed by back-calculating the calibration standards. Acceptable accuracies (- 3.1% to + 2.3%) and precision (< ± 7%) were found at concentrations of 1.00 to 500 ng/ml.

The procedure was also established at
to provide toxicokinetic (TK) monitoring for the one year rat and dog studies [D-9, D-15]. Calibration was from 1.00 to 2000 ng/ml, and assay performance was controlled by QCs prepared at 5.02, 100.4 and 1004 ng/ml. Using 1 ml of plasma the LOQ was 1.00 ng/ml, but a limit of 4.00 ng/ml was set for certain of the rat samples which were analyzed using 0.5 ml, as sufficient volume was not available for the standard assay. Subsequently, Eisai modified the method for the analysis of 0.5 ml samples of dog

END OF SPONSOR'S SUMMARY (ADME/PK)

plasma and 0.1 ml of rat plasma and these procedures were used to compare the single versus repeat dose kinetics in rats [D-2, D-11].

Modified procedures were also established by . to provide TK monitoring for the 13 week dietary studies in mice [D-13] and rats [D-10], using 0.2 ml plasma for the mouse and 0.4 ml for the rat study. Performance during TK monitoring was assessed by back-calculation of calibration standards, and controlled by the inclusion of QCs. Based on the lowest standard that could be measured to within 20% of the target concentration with an RSD of ≤20%, the LOQ was set at 20 ng/ml for the rat and 25 ng/ml for the mouse studies. The higher LOQ's in th studies may be due to a combination of the smaller volumes analyzed, plus a greater proportion of organic solvent in the eluent which decreased the elution time (< 4.0 min versus > 7.0 min at Eisai).

The assays used in various ancillary studies were also validated. For example, modifications of the HPLC-UV method were developed to support in vitro studies to determine (a) the protein binding of E2020 [D-17, D-18] and (b) the rates of formation of M1 - M6 when the drug was incubated with liver microsomes [D-20]. However, the radiochemical analysis methods are presented next.

3.9.2. QUANTIFICATION OF RADIOACTIVITY

In the studies that they performed, set the LOQ at twice the background count. In the single and repeat dose ADE study [D-12] the samples were combusted prior to analysis, which gave a background of ca. 50 dpm. The concentration which this represents, however, depends upon the specific activity of the dose and the volume/weight of sample counted. For example at the dose of 100.45 µCi/mg of E2020, the LOQ for 50 µl samples of rat blood was < 3.9 ng-eq./ml [D-12].

In the studies performed at Eisai, the concentrations of radioactivity in plasma tissues and excreta were determined by liquid scintillation counting (LSC) after solubilization, with a background count of ca. 20 dpm. In the single oral dose ADME studies the LOQ was set at 40 dpm [D-4, D-7]. As both studies were performed at a specific activity of ca. 133 µCi/mg, the LOQs for 50 µl of rat blood was 2.70 ng-eq./ml [D-4], while that for the 100 µl samples analyzed in the dog study [D-7] was 1.36 ng-eq./ml. A 40 dpm LOQ was also used for plasma (50 µl) and ultracentrifugation (100 µL) samples analyzed from the *in vitro* protein binding study [D-16], as well as for the protein binding interaction study with ^{14}C-warfarin and ^{3}H-digoxin [D-18].

ACUTE TOXICITY:

Acute toxicity studies were performed in mice (ICR), rat (Sprague-Dawley), and beagle dogs. The following results were obtained in mice and rats.

Species	Route	14 day LD 50 (mg/kg)	Highest Non-Lethal Dose (mg/kg)	Highest Non-Convulsive Dose (mg/kg)
Mouse	P.O.	M: 45	20	13
		F: 48	30	20
	I.V.	M: 3.7	2.7	2.7
		F: 4.8	3.5	2.7
Rat	P.O.	M: 37	29	35
		F: 33	24	29
	I.V.	M: 8.0	5.9	3.5
		F: 7.6	5.9	3.5

In mice, deaths occurred within 30 minutes and 5 minutes after p.o. and i.v. dosing, respectively. Toxic signs included reduced spontaneous movement (except for increases at higher i.v. doses), crouching posture, lacrimation, tremors (lower i.v. doses only), clonic convulsions, staggering gait (p.o. only), prone position, depressed respiration, and salivation. At least some signs were seen at all doses (LD = 13 and 2.1 mg/kg for p.o. and i.v., respectively). All signs in survivors resolved within 24 hours after p.o. dosing and within 4 hours after i.v. dosing. At necropsy, petechial hemorrhages were seen in lungs in all animals that died by either route; it was stated that this is consistent with hypoxia due to respiratory paralysis. Petechial hemorrhages in the glandular portion of the stomach were found in 1 of 10 mice that died after the highest oral dose (150 mg/kg). No gross pathological abnormalities were found among survivors.

In rats, most deaths after p.o. dosing occurred within 30 minutes; all by 3 days. After i.v. dosing, all deaths occurred within 30 minutes except for 1 (by 2 hours). Toxic signs included reduced spontaneous movement, staggering gait (p.o. only), tremors, clonic convulsions, lacrimation that was sometimes red, prone position, salivation, miosis, respiratory depression, and lowered surface body temperature (p.o. only). At least some signs were seen at all doses (LD = 20 and 2.1 mg/kg for p.o. and i.v., respectively). All signs in survivors resolved within 3-4 days after p.o. dosing and within 1-3 days after i.v. dosing. Necropsy of animals that died showed petechial hemorrhages in lung in 17/25 (p.o.) and 4/15 (i.v.), said to be consistent with hypoxia due to respiratory paralysis. Petechial hemorrhages in the glandular mucosa of the of the stomach were seen in 10/25 of the orally treated rats that died; "test substance-like residue" was also seen in the stomach of most orally treated rats that died. No gross pathological abnormalities were found among survivors.

In a study in dogs, 1/sex received 5, 10, or 15 mg/kg in capsules. Both HD dogs died: 1 developed fasciculation, tremors and staggering gait within 1 hour, followed by emesis, salivation, alternating pupillary constriction and dilation, clonic convulsions, and death at 2-3 hours; the other dog followed a similar course but over a longer time period. (Found dead the following day). Signs at the lower doses included fasciculations, tremors, and salivation. The MD F also had staggering gait and emesis, and persisting for 2-4 days, hypoactivity, mucoid eye discharge, miosis, and redness of the conjunctiva. Aside from this MD, most signs disappeared by 5 and 24 hours post-dose in LD and MD, respectively. Blood chemistry showed, in the MD F, elevated CPK, LDH, and AST, consistent with the prolonged fasciculations and tremors in this animal, and elevated AP, ALT, and glucose. Elevated CPK and AST were also seen in the HD F that died. (Not performed in the HD M; no effects seen in LD or in the MD M). Urinalysis showed low pH and presence of protein, glucose, and occult blood in the MD F. Histopathological exams showed no effects at LD or in the MD M. In the MD F, heart showed "myocardial degeneration and necrosis associated with interstitial edema, myocardial fibrosis, and foreign body giant cell infiltration. The degenerative and necrotic lesions containing eosinophilic myocardial fibers were multifocal and distributed in the left ventricular wall, septum, and apex". The HD dogs also showed myocardial lesions "consisting of subendocardial hemorrhage in the left papillary muscle and septum, and myocardial degeneration in the left ventricular wall and/or septum". It was stated that these lesions "were considered consistent with acute ischemic necrosis and repair, possibly due to acute hypoxia or a combination of hypoxia and local catecholamine release secondary to pharmacologically induced tremors and fasciculation". Microscopic exam of the liver showed no clear effects; brown pigment in hepatocytes was seen in the MD F. Overall results of this study are summarized in the attached table.

ACUTE DOG STUDY

Text Table 3 Summary of major toxic findings in individual animals

Dose (mg/kg)	Animal No. Sex	Clinical signs, mortality	Body weight, food and water intake	Hematology, blood-chemistry, urinalysis	Autopsy histopathology
5	8018 Male	salivation (1-2 hr) fasciculation (0-2 hr)	no toxic changes	no toxic changes	no toxic changes
5	8520 Female	fasciculation (0-3 hr) tremors (0-1 hr)	no toxic changes	no toxic changes	no toxic changes
10	8021 Male	salivation (8 hr) fasciculation (3-5 hr) tremors (3-6 hr)	no toxic changes	no toxic changes	no toxic changes
10	8521 Female	salivation (1-2 hr) fasciculation (0-10 hr) tremors (0-10 hr) staggering gait (0-1 hr) hypoactivity (23 hr-Day 4) miosis (23 hr-Day 3) redness in conjunctiva (23 hr-Day 4) mucoid eye discharge (Days 1-2)	body weight ↓ (Day 2) food intake ↓ (Days 1-3) water intake ↓ (Day 2)	platelet count ↓ (Day 3) plasma ALP, GPT, LDH ↑ (Days 1-3) GOT, CPK ↑ (6 hr-Day 3) glucose ↑ (6 hr) urinary protein, glucose, occult blood, low urine pH (Day 2)	myocardial degeneration, and necrosis, fibrosis, interstitial edema unilateral malacia
15	8022 Male	salivation, fasciculation (0-2 hr) staggering gait (0-1 hr) tremors, clonic convulsions (1-2 hr) died (2-3 hr)	NA	NA	subendocardial hemorrhage myocardial degeneration
15	8522 Female	salivation (6-8 hr) fasciculation (0-8 hr), tremors (1-8 hr) clonic convulsions (10 hr) died (10-24 hr)	NA	plasma GOT, CPK, glucose ↑ (6 hr)	subendocardial hemorrhage myocardial degeneration

Note: ↑ increase, ↓ decrease, NA: not available

Figures given in parentheses indicate time of finding after the single dose.

Study No.: 881242

3 MONTH P.O. TOXICITY IN RATS

Sprague-Dawley rats received 0, 0.3, 1, 3, 10, and 20 mg/kg/day, by gavage. N for the control and 2 highest doses was 20/sex; of these, 8/sex served as 5 week recovery groups. N for the other doses was 12/sex (no recovery groups).

Cholinergic signs were seen at 3 mg/kg and above. At 3 mg/kg fasciculations and miosis were seen in a few animals, only during the first 8 days of dosing. At the higher doses, fasciculation, hypoactivity, miosis, lacrimation, and salivation were seen; these were not seen during the recovery period. Deaths occurred in 4 and 5 rats in the 10 and 20 mg/kg groups, respectively, day 29-89; deaths were attributed to gavage errors possibly secondary to increased cholinergic tone. Bodyweight gain was decreased at 10 and 20 mg/kg; during the recovery period weight gain was greater than in controls.

Ophthalmoscopic exam, performed periodically in 4/sex/group, showed miosis or loss of pupillary reflex or soiling around the eyelid in a few rats at 10 and 20 mg/kg. Hematology and blood chemistry (done at week 13 and after recovery) showed no clear or pronounced drug effects. Slight increases in blood K were seen at the higher doses at week 13; mean values were 6-7% above control and all individual values were WNL. A few rats across all doses had elevated ALT, AST, or LDH (usually all 3 [ranging from 2 to 17x the highest concurrent control; sometimes accompanied by decreased albumin and increased globulin].) (Controls and HD were the only groups examined for liver histopathology; no drug-related effects were seen). No drug effects on plasma butyrylcholinesterase were noted. Urinalysis performed week 13 (4-23 hrs. post-dose samples) showed generally dose-related decreases in electrolyte excretion, due to decreased urinary electrolyte concentration (rather than to deceased urine volumes). (See attached table). (After recovery, Na and K excretion tended to be decreased, in F only, but not statistically significantly). Submaxillary gland weights were increased at 10 and 20 mg/kg; this was associated histologically with acinar cell hypertrophy (which was also seen at 3 mg/kg) and is consistent with cholinergic stimulation. (See attached table for incidence values). Slight to moderate mucosal edema of the forestomach was seen grossly in about 20% of the males at 10-20 mg/kg sacrificed at termination (but not after recovery); histologically this was seen in both sexes at these doses at termination (see table). (After recovery the histological incidence was slightly increased, to a much lesser degree than at termination). One HD M had forestomach erosion at termination. The only other drug-related finding was an increased incidence of slight hypertrophy of the pars intermedia of the pituitary in HD at termination (see table) but not after recovery. (Aside from the organs shown in the table + submaxillary lymph nodes and sublingual glands, histopathology was only done in controls and HD).

Overall, aside from the changes in urinary Na excretion as discussed above, 1 mg/kg was a no-effect dose in this study; 3 mg/kg was associated with transent cholinergic signs and histological evidence of increased salivation. Higher doses also caused decreased weight gain, forestomach edema (local irritating effect?), and slight hypertrophy of the pars intermedia of the pituitary (HD only); these tended to reverse after drug withdrawal. Several deaths occurred at 10 and 20 mg/kg which were attributed to gavage errors.

Text Table 4 Mean (±S.E.) urine volumes and urinary electrolyte concentrations at the end of the 13-week treatment period. Urine was collected during the period 4 to 23 hours after the final dosing

Sex	Dose	No. of animals	Urine volume	U-Na⁺		U-K⁺		U-Cl⁻	
	(mg/kg)		(ml/19 hrs)	(mEq/l)	(mEq/19 hrs)	(mEq/l)	(mEq/19 hrs)	(mEq/l)	(mEq/19 hrs)
Male	Control	12	11.8±1.02	83±9.0	0.97±0.092	128±11.7	1.41±0.085	41±4.3	0.45±0.029
	0.3	12	13.5±1.23	20±2.3**	0.25±0.025**	105±9.5	1.31±0.071	31±3.2	0.40±0.033
	1	12	11.9±1.20	20±3.6**	0.22±0.032**	115±10.7	1.25±0.057	34±4.2	0.37±0.026
	3	12	13.7±1.48	18±2.6**	0.22±0.024**	106±9.5	1.32±0.065	34±3.2	0.42±0.025
	10	12	15.4±1.88	12±1.7**	0.17±0.022**	79±8.8**	1.05±0.056**	25±2.7**	0.36±0.035
	20	12	11.4±1.34	13±2.4**	0.14±0.025**	73±6.3**	0.75±0.049**	25±9.8*	0.26±0.035**
Female	Control	11#	9.9±2.32	27±4.3	0.21±0.025	106±17.5	0.75±0.050	48±8.6	0.35±0.028
	0.3	12	8.8±1.19	26±3.5	0.20±0.021	94±8.6	0.73±0.049	41±3.2	0.33±0.027
	1	11#	8.3±1.18	24±3.9	0.19±0.030	105±12.1	0.78±0.057	45±3.8	0.35±0.033
	3	12	8.6±1.10	26±3.5	0.19±0.018	98±11.5	0.71±0.030	48±4.3	0.36±0.021
	10	9	8.8±1.83	12±3.2*	0.08±0.014**	88±20.5	0.58±0.029**	36±10.2	0.24±0.040*
	20	10	9.3±2.21	20±6.1	0.11±0.018**	78±16.5	0.47±0.029**	32±8.5	0.19±0.029**

**,* : significant difference as compared with control at $P \leq 0.01$, $0.01 < P \leq 0.05$

: The data of F002 and F037 were excluded because of water contamination.

Text Table 6 Histopathological findings in rats orally treated with E2020 for 13 weeks

Dose (mg/kg)	Category	Cont			0.3			1			3			10			20		
		M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
PITUITARY:																			
hypertrophy of pars intermedia	-	12	12	24	12	12	24	12	12	24	12	12	24	12	9	21	6	8	14
	±	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	7
SUBMAXILLARY GLANDS:																			
acinar cell hypertrophy	-	12	12	24	12	12	24	12	12	24	11	7	18	1	0	1	0	0	0
	±	0	0	0	0	0	0	0	0	0	1	5	6	5	3	8	4	7	11
	+	0	0	0	0	0	0	0	0	0	0	0	0	6	6	12	8	3	11
STOMACH:																			
edema in forestomach submucosa	-	11	12	23	10	12	22	12	12	24	11	9	20	8	7	15	7	6	13
	±	1	0	1	2	0	2	0	0	0	1	3	4	2	0	2	2	0	2
	+	0	0	0	0	0	0	0	0	0	0	0	0	2	2	4	3	4	7
erosion in forestomach	-	12	12	24	12	12	24	12	12	24	12	12	24	12	9	21	11	10	21
	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1

Category: findings were graded as,

-: no change, ±: slight, +: moderate, ++: marked

M: male, F: female, T: total

#: The pituitary of F083 was lost during the embedding procedure.

ONE YEAR P.O. TOXICITY IN RATS:

A) Dosage

40/sex at 0, 1, 3, or 10 mg/kg/day, by gavage

10/sex/group were sacrificed at 6 months. Attached table indicates numbers of animals used for lab tests, PK evaluation, and pathology exams.

Strain: Charles River CD

Drug Lot #s: 88020201
89120401

B) Results

1) Observed signs

a) Miosis (bilateral) was the most frequently observed sign. Seen at all doses, generally D-R earlier in the study; incidence decreased and became generally non D-R during second half of study.

b) Other signs, of relatively low incidence, increased mainly at HD: anogenital staining, fasciculations, soft stool (M only, generally in first 3 months), tail bumps (M only; histopath = hyperkeratosis), and alopecia.

2) Mortality

No drug effects.

A total of 30 rats died or were prematurely sacrificed; see attached table.

3) Bodyweight gain

Decreased at HD; final weight 11% and 15% below control in M and F, resp. (See attached figures).

4) Food Consumption

Drug effects cannot be accurately determined from the summary table provided since food consumption was expressed as grams consumed per kg bodyweight. However, in view of the statistically significantly decreased values in these units at HD during the first 1-2 weeks of treatment (times when bodyweights were slightly less than control) it may be inferred that food consumption per animal was decreased during this time. Also, since g/kg food consumption in HD M during the first half of the study was often similar to controls, it may be inferred that food consumption per animal was decreased to the same degree as was the bodyweight during this time.

5) Ophthalmoscopic exam

(Done pre-Tx and months 3, 6, 12)

No drug effects

6) Hematology

(Done at 6 months and termination)

No clear drug effects. Two (of 23) HD M had relatively low numbers of lymphocytes and relatively high numbers of neutrophils (with normal total WBC) at termination.

Parameters measured: RBC, Hb, Hct, RBC morphology, reticulocytes, platelets, PT, APTT, WBC, differential.

7) Blood chemistry

(Done at 6 months and termination)

No clear drug effects. A few statistically significant differences in mean values were noted; these were quite small, with individual values generally not greatly outside the control range, and included the following:

a) Increased BUN in HD M (with a trend in LD and MD M) at 6 months and in HD F at termination.

b) Decreased glucose in HD F at 6 months and termination. (Glucose was increased in LD M and MD M at termination).

c) Decreased total protein in HD M at termination.

d) Decreased triglycerides in HD M at termination.

e) Increased phospholipids in HD F at termination.

f) Increased phosphorus in MD and HD M at termination.

Other parameters measured: ALT, AST, AP, LDH, total bilirubin, albumin, protein electrophoresis, CPK, creatinine, cholesterol, Na, K, Cl, Ca.

8) Urinalysis

(Done at months 3 and 6 at termination. At 3 months urine volume, electrolyte concentrations, and electrolyte excretion was studied at both 0-4 hr. and 4-24 hr. post-dosing).

At 3 months, 0-4 hr. time period, urinary concentrations of Na, K and Cl were increased in all M groups (not clearly D-R), urine volume was increased in HD M, and urinary excretion of Na, K and Cl increased in MD and HD M (D-R). (Mean electrolyte excretion values at HD were about 2-4x control). Similar but less pronounced effects wee seen in F at this time point: Na and Cl concentrations were slightly increased at HD, urine volume was equivocally increased at MD and HD, and urinary excretion Na, K, and Cl was increased at HD. (Na at MD also). (Mean electrolyte excretion values in HD F ~ 1.5 - 2x control). The above effects were not seen during the 4-24 hr. time period. For the overall 0-24 hr. period, excretion of Na, K, and Cl were slightly increased in MD and HD M (D-R; means at HD ~ 1.4x control), with no effect in F.

At the 6 month measurement (24 hr. measurement only) K concentration was slightly increased in HD M, and Na, K, and Cl concentrations were slightly increased in HD F, but there were no effects on electrolyte excretion (since urine volume was slightly below control).

At the 12 month measurement (24 hr. measurement only) electrolyte concentrations (except for Na in females) were slightly increased at HD; however urine volume was slightly decreased at HD resulting in no drug effects of electrolyte excretion.

8) Plasma levels of parent drug

Samples were taken from 5/sex/group, 1 and 4 hours post-dose, on the first and third days of weeks 1, 5, 27, and 52. Samples were pooled (by dose, gender, day, and time); results for the 1 hour time point are shown in the attached table. (Values at the 4 hour time point were ~ 1/2 those at the 1 hour point). Levels increased roughly proportionally to dose. Levels also increased with increased duration of dosing. Levels tended to be greater in F than in M at most points.

9) Organ weights

Absolute and relative salivary gland weight increased at HD at both 6 month and terminal sacrifices. (Meal rel. wt. ~ 1.25x control). (No associated histopathology).

10) Gross pathology

No summary tables presented. Text says no drug effects.

11) Microscopic pathology

(Routine exam done in control, HD, and premature deaths. [See table under "Dosage", above for numbers examined at 6 month and terminal sacrifices]. Gross lesions examined histologically in all groups).

No drug effects. There was an equivocal trend toward decreased chronic nephropathy in HD M.

(SPONSOR'S SUMMARY TABLES ATTACHED)

3. METHODS (cont.): 10

E. Experimental Outline:

Group	Test Substance	Dose Level[a] (mg/kg/day)	Number of Animals: Total Initial M	F	Pharmacokinetic Study[b] M	F	Clinical Laboratory Studies[c] Mo. 3 M	F	Mo. 6 M	F	Mo. 12 M	F	Necropsy Mo. 6 M	F	Mo. 12 M	F	Histopathology[d] Mo. 6 M	F	Mo. 12 M	F
I	Vehicle	0	40	40	5	5	10	10	10	10	18	23	10	10	18	23	12	11	24	24
II	E2020	1.0	40	40	5	5	10	10	10	10	21	23	10	10	21	23	2	1	4	1
III	E2020	3.0	40	40	5	5	10	10	10	10	22	23	10	10	22	23	?	2	1	0
IV	E2020	10.0	40	40	5	5	10	10	9[e]	10	23	22	9[e]	10	23	22	11	13	24	23

[a] Doses given represent doses of the test substance as supplied by the sponsor, i.e., no correction for percent active ingredient was made.

[b] Blood plasma was collected from each rat 1 and 4 hours after dosing on the first and third day of dosing. This sampling procedure was performed on the same animals at Months 1, 6 and 12 (termination).

[c] Includes hematology, biochemistry and urinalysis. Only urinalysis was performed at the Month 3 interval. The pharmacokinetic animals were not sampled for these parameters.

[d] Histopathological evaluations of selected tissues were conducted for all animals in the control group and the high-dose group that were sacrificed at the Month 6 interim and terminal sacrifices and on any animal found dead, killed accidentally or euthanized in extremis prior to the scheduled sacrifice interval including these animals designated for pharmacokinetic analyses.

[e] One Group IV male, designated to be sacrificed at Month 6, died prior to the scheduled sacrifice interval.

	MALE					FEMALE				
GROUP	I	II	III	IV	TOTAL	I	II	III	IV	TOTAL
Terminal Sacrifice	18	21	22	23	84	23	23	23	22	91
Spontaneous Death	7	5	3	3	18	1	2	1	1	5
Sacrificed Moribund	0	0	0	0	0	1	0	0	0	1
Accidental Death	1	1	0	0	2	0	0	1	3	4
Interim Sacrifice	10	10	10	9	39	10	10	10	10	40
Pharmacokinetic	4	3	5	5	17	5	5	5	4	19
TOTAL	40	40	40	40	160	40	40	40	40	160

Figure 1
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

Group Mean Body Weight Values in Male Rats

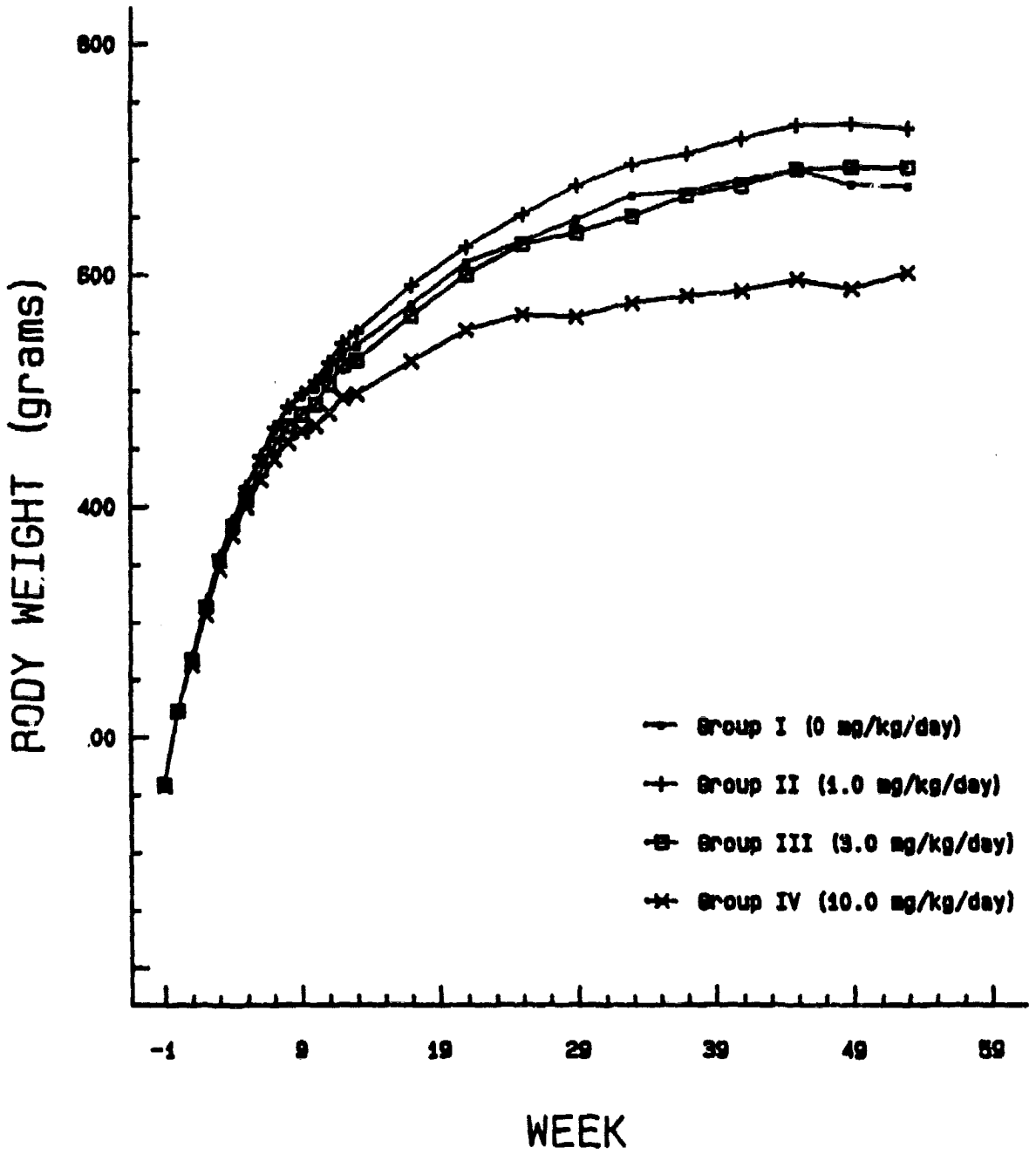
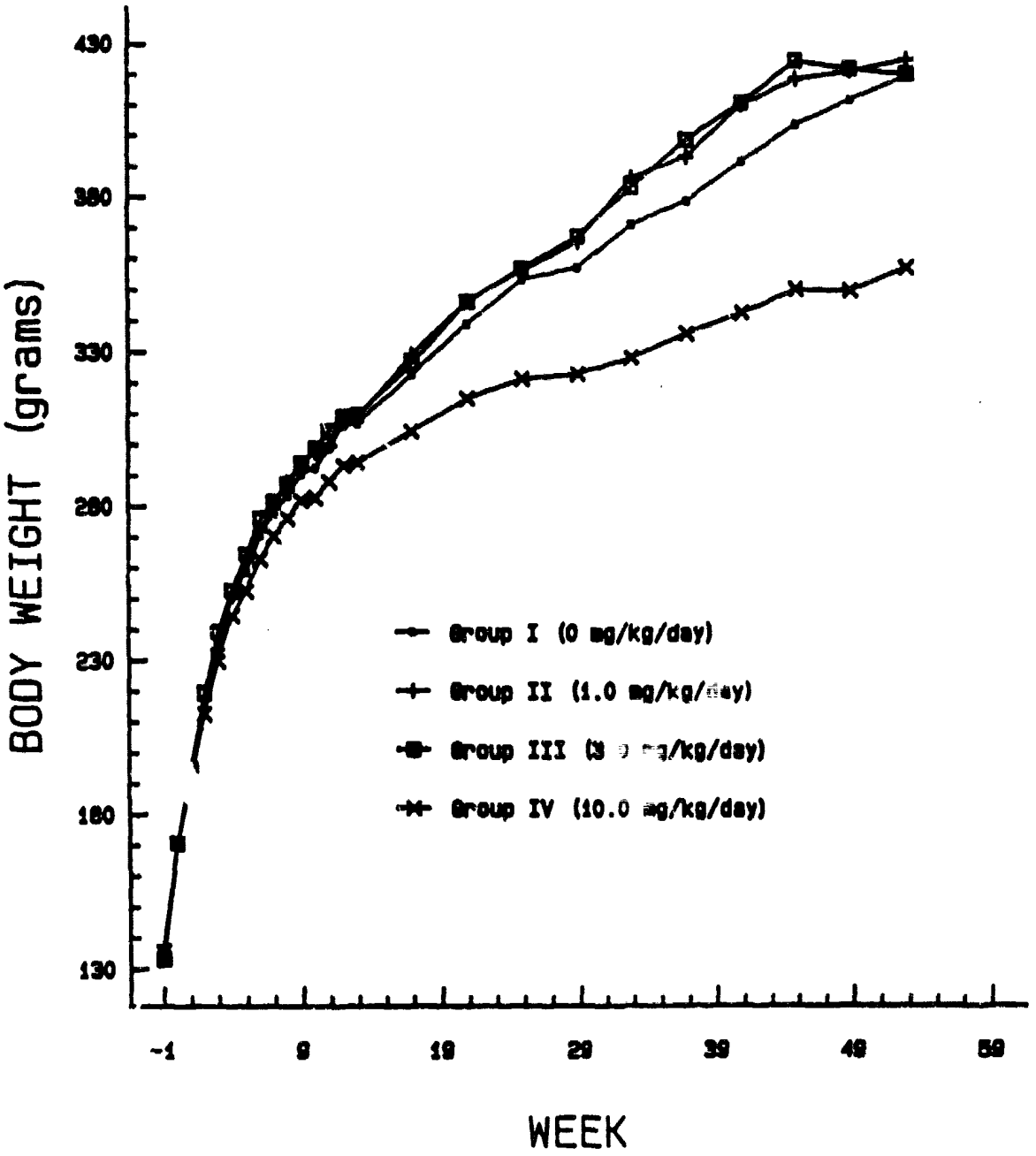


Figure 2
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

Group Mean Body Weight Values in Female Rats



11 d

Table 12: Concentrations of E2020 in plasma 1 hour after administration by gavage to male (M) and female (F) rats during a 1 year toxicity study.

Study week	Day	Dosage Group 1.0 mg/kg/day		3.0 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F
1	1	7.42	10.02	24.10	57.10	61.73	85.68
	3	13.98	11.19	32.33	55.32	92.09	104.22
5	1	6.85	17.33	40.99	75.00	157.07	159.81
	3	10.39	16.21	41.34	71.30	187.53	236.38
27	1	13.71	22.03	54.45	104.00	189.24	239.45
	3	10.58	18.82	47.17	63.93	149.50	210.33
52	1	12.95	23.95	65.34	108.42	224.06	304.64
	3	19.87	25.92	87.39	100.75	233.66	241.97

T 25A-1
Table 25A
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

Incidence of Neoplasms - Male Rats
6 Month Interim Sacrifice

Key

SC = Spinal Cord
LN = Lymph Node
GL = Gland
GLS = Glands

11 f

Table 12: Concentrations of E2020 in plasma 1 hour after administration by gavage to male (M) and female (F) rats during a 1 year toxicity study.

Study week	Day	Dosage Group 1.0 mg/kg/day		3.0 mg/kg/day		10 mg/kg/day	
		M	F	M	F	M	F
1	1	7.42	10.02	24.10	57.10	61.73	85.68
	3	13.98	11.19	32.33	55.32	92.09	104.22
5	1	6.85	17.33	40.99	75.00	157.07	159.81
	3	10.39	16.21	41.34	71.30	187.53	236.38
27	1	13.71	22.03	54.45	104.00	189.24	239.45
	3	10.58	18.82	47.17	63.93	149.50	210.33
52	1	12.95	23.95	65.34	108.42	224.06	304.64
	3	19.87	25.92	87.39	100.75	233.66	241.97

T 25A-1
Table 25A
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Incidence of Neoplasms - Male Rats
6 Month Interim Sacrifice

Key

SC = Spinal Cord
LN = Lymph Node
GL = Gland
GLS = Glands

T 27A-1
Table 27A
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Non-Neoplastic Lesion Incidence - Male Rats
6 Month Interim Sacrifice

Key

U/ = Unilateral
B/ = Bilateral
SC = Spinal Cord
LN = Lymph Node
GL = Gland
GLS = Glands

11 dd

T 27A-2
Table 27A (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	9
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	10	0	0	9
CERVICAL SC	NUMBER EXAMINED:	9	0	0	9
THORACIC SC	NUMBER EXAMINED:	10	0	0	9
LUMBAR SC	NUMBER EXAMINED:	10	0	0	9
NERVE	NUMBER EXAMINED:	10	0	0	9
HEART	NUMBER EXAMINED:	10	0	0	9
SALIVARY GLANDS	NUMBER EXAMINED:	10	0	0	9
AORTA	NUMBER EXAMINED:	10	0	0	9
TRACHEA	NUMBER EXAMINED:	10	0	0	9
--ACUTE/SUBACUTE INFLAMMATION		1	0	0	0
ESOPHAGUS	NUMBER EXAMINED:	10	0	0	9
LUNGS	NUMBER EXAMINED:	10	6	5	9
--CHRONIC INTERSTITIAL PNEUMONIA		9	6	4	9
--CONGESTION		1	0	0	0
--HEMORRHAGE		0	4	0	2
--EMPHYSEMA		5	6	4	5
--PURULENT BRONCHOPNEUMONIA		1	0	0	0
--ACUTE PLEURITIS/ADHESIONS		1	0	0	0
--ABSCESS(ES)		1	0	0	0
MESENTERIC LN	NUMBER EXAMINED:	9	0	0	9
MEDIASTINAL LN	NUMBER EXAMINED:	10	0	1	9
--LYMPHOID CELL HYPERPLASIA		2	0	0	1
--CONGESTION/HEMORRHAGE		0	0	1	1
THYMUS	NUMBER EXAMINED:	10	0	0	9
SPLEEN	NUMBER EXAMINED:	10	0	0	9

T 27A-3
Table 27A (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	9
LIVER	NUMBER EXAMINED:	10	0	0	9
--TENSION LIPIDOSIS		1	0	0	1
BONE STERNUM	NUMBER EXAMINED:	10	0	0	9
BONE FEMUR	NUMBER EXAMINED:	10	0	0	9
BONE RIB	NUMBER EXAMINED:	7	0	0	6
MARROW STERNUM	NUMBER EXAMINED:	10	0	0	9
MARROW FEMUR	NUMBER EXAMINED:	10	0	0	9
MARROW RIB	NUMBER EXAMINED:	7	0	0	8
STOMACH	NUMBER EXAMINED:	10	0	0	9
DUODENUM	NUMBER EXAMINED:	10	0	0	9
PANCREAS	NUMBER EXAMINED:	10	0	0	9
--ACINAR ATROPHY		0	0	0	1
--STEATOSIS		0	0	0	1
JEJUNUM	NUMBER EXAMINED:	10	0	0	9
ILEUM	NUMBER EXAMINED:	10	0	0	9
CECUM	NUMBER EXAMINED:	10	0	0	9
COLON	NUMBER EXAMINED:	10	0	0	9
--LUMEN:NEMATODES		0	0	0	1
RECTUM	NUMBER EXAMINED:	10	0	0	9
--LUMEN:NEMATODES		3	0	0	1
TONGUE	NUMBER EXAMINED:	8	0	0	7
KIDNEYS	NUMBER EXAMINED:	10	1	2	9
--B/CHRONIC NEPHROPATHY		6	1	0	1

** CONTINUED ON NEXT PAGE **

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	9
** FROM PREVIOUS PAGE **					
KIDNEYS	NUMBER EXAMINED:	10	1	2	9
--U/HYDRONEPHROSIS		2	0	0	1
--B/HYDRONEPHROSIS		0	0	1	0
URINARY BLADDER	NUMBER EXAMINED:	9	0	0	8
TESTES	NUMBER EXAMINED:	10	0	0	9
EPIDIDYMIDES	NUMBER EXAMINED:	10	0	0	9
PROSTATE	NUMBER EXAMINED:	10	0	0	9
SEMINAL VESICLES	NUMBER EXAMINED:	10	1	0	9
COAGULATING GL.	NUMBER EXAMINED:	10	0	0	9
PITUITARY GLAND	NUMBER EXAMINED:	9	0	0	9
THYROID GLAND	NUMBER EXAMINED:	9	0	0	9
--THYROGLOSSAL CYST(S)		2	0	0	2
PARATHYROID GLS.	NUMBER EXAMINED:	9	0	0	7
ADRENAL GLANDS	NUMBER EXAMINED:	10	0	0	9
--B/CORTEX:VACUOLATION		1	0	0	0
EYES	NUMBER EXAMINED:	10	0	0	9
--U/RETROBULBAR HEMORRHAGE		2	0	0	5
HARDERIAN GLANDS	NUMBER EXAMINED:	10	0	0	9
MUSCLE	NUMBER EXAMINED:	10	0	0	9
SKIN	NUMBER EXAMINED:	10	2	1	9
--ABSCESS(ES)		0	1	0	0
--ACUTE INFLAMMATION/NECROSIS		0	1	0	0
LYMPH NODE	NUMBER EXAMINED:	0	1	0	1
--LYMPHOID CELL HYPERPLASIA		0	1	0	0

** CONTINUED ON NEXT PAGE **

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	9
** FROM PREVIOUS PAGE **					
LYMPH NODE	NUMBER EXAMINED:	0	1	0	1
--CONGESTION/HEMORRHAGE		0	0	0	1
--PLASMA CELL HYPERPLASIA		0	0	0	1
TAIL	NUMBER EXAMINED:	0	1	1	1
--HYPERKERATOSIS		0	1	1	1
--ACUTE INFLAMMATION/NECROSIS		0	0	1	0
** END OF LIST **					

T 27B-1
Table 27B
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Non-Neoplastic Lesion Incidence - Male Rats
Terminal Sacrifice

Key

U/ = Unilateral
B/ = Bilateral
SC = Spinal Cord
LN = Lymph Node
GL = Gland
GLS = Glands

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		SEX:	MALE			
		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	18	21	22	23
** TOP OF LIST **						
BRAIN	NUMBER EXAMINED:		18	0	0	23
CERVICAL SC	NUMBER EXAMINED:		18	0	0	23
THORACIC SC	NUMBER EXAMINED:		18	0	0	23
LUMBAR SC	NUMBER EXAMINED:		18	0	0	23
NERVE	NUMBER EXAMINED:		17	0	0	23
HEART	NUMBER EXAMINED:		18	0	0	23
--MYOCARDIUM:HEMORRHAGE			0	0	0	1
--CHRONIC MYOCARDITIS/FIBROSIS			1	0	0	0
SALIVARY GLANDS	NUMBER EXAMINED:		18	0	0	23
AORTA	NUMBER EXAMINED:		18	0	0	22
TRACHEA	NUMBER EXAMINED:		18	0	0	23
ESOPHAGUS	NUMBER EXAMINED:		18	0	0	23
LUNGS	NUMBER EXAMINED:		18	4	2	23
--CHRONIC INTERSTITIAL PNEUMONIA			5	3	1	7
--EMPHYSEMA			11	2	1	8
--GRANULOMA(S)/GRANULOMATOUS INFLAMMATION			1	0	0	1
--ATELECTASIS			2	1	1	0
--CHRONIC PLEURITIS/ADHESIONS			0	0	0	1
MESENTERIC LN	NUMBER EXAMINED:		18	0	0	23
--CYST(S)			0	0	0	1
MEDIASTINAL LN	NUMBER EXAMINED:		17	0	1	23
THYMUS	NUMBER EXAMINED:		17	0	0	18
--LYMPHOID CELL ATROPHY			3	0	0	7
SPLEEN	NUMBER EXAMINED:		18	0	0	23
--LYMPHOID CELL HYPERPLASIA			1	0	0	1

** CONTINUED ON NEXT PAGE **

T 27B-2
Table 27B (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=1

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
	SEX:	-------MALE-------			
	GROUP:	-1-	-2-	-3-	-4-
	NUMBER:	21	21	22	23
** FROM PREVIOUS PAGE **					
SPLEEN	NUMBER EXAMINED:	18	0	0	23
--EXTRAMEDULLARY HEMATOPOIESIS		0	0	0	2
LIVER	NUMBER EXAMINED:	18	3	0	23
--HEPATOCELLULAR VACUOLATION		2	1	0	2
--TENSION LIPIDOSIS		0	0	0	1
--COAGULATIVE NECROSIS		1	0	0	0
--ACUTE INFLAMMATION		1	0	0	0
BONE STERNUM	NUMBER EXAMINED:	18	0	0	23
BONE FEMUR	NUMBER EXAMINED:	18	0	0	23
BONE RIB	NUMBER EXAMINED:	17	0	0	19
MARROW STERNUM	NUMBER EXAMINED:	18	0	0	23
MARROW FEMUR	NUMBER EXAMINED:	18	0	0	23
MARROW RIB	NUMBER EXAMINED:	16	0	0	20
STOMACH	NUMBER EXAMINED:	18	0	1	23
--FORESTOMACH:SQUAMOUS CELL HYPERPLASIA		0	0	0	1
--FORESTOMACH:ACUTE INFLAMMATION		0	0	0	1
--FORESTOMACH,SUBMUCOSA:EDEMA		0	0	0	1
DUODENUM	NUMBER EXAMINED:	18	0	0	23
PANCREAS	NUMBER EXAMINED:	18	0	0	23
--ACINAR ATROPHY		0	0	0	0
--STEATOSIS		2	0	0	1
--ISLET CELL HYPERPLASIA		1	0	0	1
--ACINAR CELL HYPERTROPHY		1	0	0	0
--SUBACUTE/CHRONIC INFLAMMATION		1	0	0	1
JEJUNUM	NUMBER EXAMINED:	18	0	0	23
ILEUM	NUMBER EXAMINED:	18	0	0	23

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=1

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		18	21	22	23
CECUM	NUMBER EXAMINED:	18	0	1	23
--ULCER(S)		0	0	0	1
COLON	NUMBER EXAMINED:	18	0	0	23
--LUMEN:NEMATODES		2	0	0	2
RECTUM	NUMBER EXAMINED:	18	0	0	23
--LUMEN:NEMATODES		2	0	0	6
TONGUE	NUMBER EXAMINED:	18	0	0	23
KIDNEYS	NUMBER EXAMINED:	18	3	2	23
--U/CHRONIC NEPHROPATHY		1	1	0	0
--B/CHRONIC NEPHROPATHY		14	2	2	10
--U/HYDRONEPHROSIS		0	0	0	1
--U/ACUTE PYELONEPHRITIS		0	0	0	1
--B/ACUTE PYELONEPHRITIS		0	0	0	1
--U/PELVIS:UROLITH(S)		1	0	1	1
--B/PELVES:UROLITHS		0	0	0	1
--U/ABSCESS(ES)		0	0	0	1
URINARY BLADDER	NUMBER EXAMINED:	16	0	0	22
--ACUTE/SUBACUTE INFLAMMATION		0	0	0	1
--LUMEN:PROTEINACEOUS MATERIAL		0	0	0	2
TESTES	NUMBER EXAMINED:	16	1	1	23
--U/DEGENERATION OF GERMINAL EPITHELIUM		3	0	1	1
--B/DEGENERATION OF GERMINAL EPITHELIUM		0	1	0	0
--U/SEMINIFEROUS TUBULES:MINERALIZATION		1	0	0	0
--U/INTERSTITIAL CELL HYPERPLASIA		0	0	0	1
EPIDIDYMIDES	NUMBER EXAMINED:	18	1	1	23
--U/OLIGOSPERMIA		1	0	1	1
--B/OLIGOSPERMIA		0	1	0	0
PROSTATE	NUMBER EXAMINED:	18	0	1	23
--ACUTE INFLAMMATION/NECROSIS		1	0	1	2
SEMINAL VESICLES	NUMBER EXAMINED:	18	0	0	23

T 27B-5
Table 27B (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED --- SEX: -------MALE------- GROUP: -1- -2- -3- -4- NUMBER: 18 21 22 23
COAGULATING GL.	NUMBER EXAMINED:	18 0 0 23
PITUITARY GLAND	NUMBER EXAMINED:	16 3 1 20
--CYST(S)		1 0 0 0
--PARS DISTALIS:HYPERPLASIA/HYPERTROPHY		0 0 0 1
--HEMORRHAGE		0 2 0 0
THYROID GLAND	NUMBER EXAMINED:	17 1 0 23
--THYROGLOSSAL CYST(S)		2 0 0 5
PARATHYROID GLS.	NUMBER EXAMINED:	15 0 0 22
ADRENAL GLANDS	NUMBER EXAMINED:	18 2 3 23
--B/CORTEX:VACUOLATION		1 0 0 0
--B/CORTEX:VACUOLATION		0 0 0 1
--U/ACCESSORY ADRENAL TISSUE		0 0 0 1
--U/HEMORRHAGE/HEMATOCYSTS		1 0 0 0
--B/HEMORRHAGE/HEMATOCYSTS		0 0 0 2
EYES	NUMBER EXAMINED:	18 0 0 23
--U/RETROBULBAR HEMORRHAGE		4 0 0 7
HARDERIAN GLANDS	NUMBER EXAMINED:	18 0 0 23
MUSCLE	NUMBER EXAMINED:	18 0 0 23
SKIN	NUMBER EXAMINED:	18 1 1 23
MAMMARY	NUMBER EXAMINED:	8 0 0 11
LYMPH NODE	NUMBER EXAMINED:	1 0 0 0
--PLASMA CELL HYPERPLASIA		1 0 0 0
TAIL	NUMBER EXAMINED:	3 2 1 6
--HYPERKERATOSIS		3 2 1 5
--ACUTE INFLAMMATION/NECROSIS		3 0 0 1
EARS	NUMBER EXAMINED:	0 2 0 1
--B/CHONDROID CELL HYPERPLASIA		0 1 0 1

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
	SEX:	-------MALE-------			
	GROUP:	-1-	-2-	-3-	-4-
	NUMBER:	18	21	22	23
EXTREMITY	NUMBER EXAMINED:	1	0	0	4
--CHRONIC ACTIVE INFLAMMATION		1	0	0	4
PREPUTIAL GLAND	NUMBER EXAMINED:	0	0	0	3
--ABSCESS(ES)		0	0	0	1
--ACUTE/SUBACUTE INFLAMMATION		0	0	0	2

** END OF LIST **

T 278-6
Table 27B (cont.)

T 27C-1
Table 27C
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Non-Neoplastic Lesion Incidence - Male Rats
Unscheduled Deaths

Key

U/ = Unilateral
B/ = Bilateral
SC = Spinal Cord
LN = Lymph Node
GL = Gland
GLS = Glands
LYMPH/RET. NEOPL. = Lymphoreticular Neoplasm

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T 27C-2
Table 27C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		SEX:	MALE			
		GROUP:	-1-	-2-	-3-	-4-
			--- NUMBER OF ANIMALS AFFECTED ---			
	NUMBER:		8	6	3	3
** TOP OF LIST **						
BRAIN	NUMBER EXAMINED:		8	6	3	3
CERVICAL SC	NUMBER EXAMINED:		8	6	3	3
THORACIC SC	NUMBER EXAMINED:		8	6	3	3
--HEMORRHAGE			0	1	0	0
LUMBAR SC	NUMBER EXAMINED:		8	6	3	3
NERVE	NUMBER EXAMINED:		8	6	3	3
HEART	NUMBER EXAMINED:		8	6	3	3
--CHRONIC MYOCARDITIS/FIBROSIS			1	0	0	0
SALIVARY GLANDS	NUMBER EXAMINED:		7	6	3	3
AORTA	NUMBER EXAMINED:		8	6	3	3
TRACHEA	NUMBER EXAMINED:		8	6	3	3
--HEMORRHAGE			0	0	0	1
ESOPHAGUS	NUMBER EXAMINED:		8	6	3	3
LUNGS	NUMBER EXAMINED:		8	6	3	3
--CHRONIC INTERSTITIAL PNEUMONIA			2	4	0	1
--CONGESTION			7	6	3	3
--HEMORRHAGE			2	2	1	0
--EDEMA			1	0	0	0
--EMPHYSEMA			3	2	2	1
--ACUTE BRONCHITIS/PERIBRONCHITIS			0	0	0	1
--INTRABRONCHIAL FOREIGN BODIES			0	0	0	1
--ALVEOLAR MACROPHAGES:ACCUMULATION			1	0	0	1
--GRANULOMA(S)/GRANULOMATOUS INFLAMMATION			0	0	0	1
--ATELECTASIS			1	0	0	0
--ACUTE BRONCHOPNEUMONIA			1	0	0	0
MESENTERIC LN	NUMBER EXAMINED:		8	6	3	3
--CONGESTION/HEMORRHAGE			1	0	0	0

T 27C-3
Table 27C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		8	6	3	3
MEDIASTINAL LN	NUMBER EXAMINED:	8	6	3	3
--LYMPHOID CELL HYPERPLASIA		0	1	0	0
--CONGESTION/HEMORRHAGE		3	4	1	1
--RETICULOENDOTHELIAL CELL HYPERPLASIA		0	1	0	0
THYMUS	NUMBER EXAMINED:	7	6	2	2
--LYMPHOID CELL ATROPHY		3	0	1	0
--CONGESTION/HEMORRHAGE		2	0	0	1
SPLEEN	NUMBER EXAMINED:	8	6	3	3
--RETICULOENDOTHELIAL CELLS: BROWN PIGMENT		1	0	0	1
--CONGESTION/HEMORRHAGE		1	0	0	0
LIVER	NUMBER EXAMINED:	8	6	3	3
--HEPATOCELLULAR VACUOLATION		2	2	1	0
--CONGESTION		8	5	3	3
--HEMORRHAGE		1	1	0	0
--COAGULATIVE NECROSIS		2	0	0	0
BONE STERNUM	NUMBER EXAMINED:	8	6	3	3
BONE FEMUR	NUMBER EXAMINED:	8	6	3	3
BONE RIB	NUMBER EXAMINED:	2	4	2	3
MARROW STERNUM	NUMBER EXAMINED:	8	6	3	3
MARROW FEMUR	NUMBER EXAMINED:	8	6	3	3
MARROW RIB	NUMBER EXAMINED:	2	4	2	3
STOMACH	NUMBER EXAMINED:	7	6	3	3
DUODENUM	NUMBER EXAMINED:	8	6	3	3
PANCREAS	NUMBER EXAMINED:	8	6	3	3
--STEATOSIS		1	0	0	0
JEJUNUM	NUMBER EXAMINED:	8	6	2	2

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		8	6	3	3
ILEUM	NUMBER EXAMINED:	5	6	2	2
CECUM	NUMBER EXAMINED:	7	6	2	2
--LUMEN:NEMATODES		0	1	0	0
COLON	NUMBER EXAMINED:	8	6	3	2
--LUMEN:NEMATODES		1	2	1	0
RECTUM	NUMBER EXAMINED:	8	6	3	3
--LUMEN:NEMATODES		0	2	1	0
TONGUE	NUMBER EXAMINED:	8	6	3	3
KIDNEYS	NUMBER EXAMINED:	8	6	3	3
--B/CHRONIC NEPHROPATHY		2	3	0	0
--B/HYDRONEPHROSIS		0	1	0	0
--B/CONGESTION		6	4	3	3
--U/CORTICAL CYST(S)		0	1	0	0
--B/ACUTE PYELONEPHRITIS		1	0	0	0
URINARY BLADDER	NUMBER EXAMINED:	8	6	3	3
--ACUTE/SUBACUTE INFLAMMATION		1	1	0	0
--UROTHELIAL HYPERPLASIA		1	1	0	0
--LUMEN:PROTEINACEOUS MATERIAL		0	1	0	0
--LUMEN:DILATION		2	0	0	0
--LUMEN:UROLITHS		1	0	0	0
TESTES	NUMBER EXAMINED:	8	6	3	3
EPIDIDYMIDES	NUMBER EXAMINED:	8	6	3	3
--B/OLIGOSPERMIA		0	1	0	0
PROSTATE	NUMBER EXAMINED:	7	6	3	3
--ACUTE INFLAMMATION/NECROSIS		1	1	0	0
SEMINAL VESICLES	NUMBER EXAMINED:	8	6	3	3
--ACUTE INFLAMMATION/NECROSIS		1	1	0	0

T 27C-5
Table 27C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		8	6	3	3
COAGULATING GL.	NUMBER EXAMINED:	8	6	3	3
--ACUTE INFLAMMATION/NECROSIS		1	1	0	0
PITUITARY GLAND	NUMBER EXAMINED:	8	6	3	3
--CYST(S)		0	1	2	1
THYROID GLAND	NUMBER EXAMINED:	8	6	2	2
PARATHYROID GLS.	NUMBER EXAMINED:	6	4	3	2
ADRENAL GLANDS	NUMBER EXAMINED:	8	6	3	2
--B/CORTEX:VACUOLATION		1	0	0	0
--B/CONGESTION		4	1	2	1
--U/ACCESSORY ADRENAL TISSUE		0	0	0	1
EYES	NUMBER EXAMINED:	8	6	3	3
HARDERIAN GLANDS	NUMBER EXAMINED:	8	6	3	3
MUSCLE	NUMBER EXAMINED:	8	6	3	3
SKIN	NUMBER EXAMINED:	8	6	2	3
MAMMARY	NUMBER EXAMINED:	0	1	0	0
LYMPH NODE	NUMBER EXAMINED:	2	3	0	0
--LYMPHOID CELL HYPERPLASIA		0	1	0	0
--CONGESTION/HEMORRHAGE		1	2	0	0
TAIL	NUMBER EXAMINED:	1	0	1	1
--HYPERKERATOSIS		1	0	1	1
--ACUTE INFLAMMATION/NECROSIS		0	0	1	1
EARS	NUMBER EXAMINED:	1	0	0	1
--U/CHRONIC ACTIVE INFLAMMATION		0	0	0	1
NOSE/TURBINATES	NUMBER EXAMINED:	1	1	0	1
--CONGESTION		0	0	0	1

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		8	6	3	3
PREPUTIAL GLAND	NUMBER EXAMINED:	0	0	0	1
--ABSCESS(ES)		0	0	0	1
BONE	NUMBER EXAMINED:	0	1	0	0
--FRACTURE		0	1	0	0
--HEMORRHAGE		0	1	0	0
LYMPHO/RET.NEOPL	NUMBER EXAMINED:	1	0	0	0
PENIS	NUMBER EXAMINED:	1	0	0	0
--CONGESTION		1	0	0	0

** END OF LIST **

T 25C-1
Table 25C
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Incidence of Neoplasms - Male Rats
Unscheduled Deaths

Key

U/ = Unilateral
M- = Malignant
SC = Spinal Cord
LN = Lymph Node
GL = Gland
GLS = Glands
MESEN/PERITONEUM = Mesentery/Peritoneum
LYMPH/RET. NEOPL. = Lymphoreticular Neoplasm

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED --- SEX: MALE			
		GROUP: -1-	-2-	-3-	-4-
	NUMBER:	8	6	3	3
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	8	6	3	3
CERVICAL SC	NUMBER EXAMINED:	8	6	3	3
THORACIC SC	NUMBER EXAMINED:	8	6	3	3
LUMBAR SC	NUMBER EXAMINED:	8	6	3	3
NERVE	NUMBER EXAMINED:	8	6	3	3
HEART	NUMBER EXAMINED:	8	6	3	3
SALIVARY GLANDS	NUMBER EXAMINED:	7	6	3	3
AORTA	NUMBER EXAMINED:	8	6	3	3
TRACHEA	NUMBER EXAMINED:	8	6	3	3
ESOPHAGUS	NUMBER EXAMINED:	8	6	3	3
LUNGS	NUMBER EXAMINED:	8	6	3	3
MESENTERIC LN	NUMBER EXAMINED:	8	6	3	3
MEDIASTINAL LN	NUMBER EXAMINED:	8	6	3	3
THYMUS	NUMBER EXAMINED:	7	6	2	2
SPLEEN	NUMBER EXAMINED:	8	6	3	3
LIVER	NUMBER EXAMINED:	8	6	3	3
BONE STERNUM	NUMBER EXAMINED:	8	6	3	3
BONE FEMUR	NUMBER EXAMINED:	8	6	3	3
BONE RIB	NUMBER EXAMINED:	2	4	2	3
MARROW STERNUM	NUMBER EXAMINED:	8	6	3	3

T 25C-2
Table 25C (cont.)

T 25C-3
Table 25C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		-------MALE ------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		8	6	3	3
MARROW FEMUR	NUMBER EXAMINED:	8	6	3	3
MARROW RIB	NUMBER EXAMINED:	2	4	2	3
STOMACH	NUMBER EXAMINED:	7	6	3	3
DUODENUM	NUMBER EXAMINED:	8	6	3	3
PANCREAS	NUMBER EXAMINED:	8	6	3	3
JEJUNUM	NUMBER EXAMINED:	8	6	2	2
ILEUM	NUMBER EXAMINED:	5	6	2	2
CECUM	NUMBER EXAMINED:	7	6	2	2
COLON	NUMBER EXAMINED:	8	6	3	2
RECTUM	NUMBER EXAMINED:	8	6	3	3
TONGUE	NUMBER EXAMINED:	8	6	3	3
KIDNEYS	NUMBER EXAMINED:	8	6	3	3
URINARY BLADDER	NUMBER EXAMINED:	8	6	3	3
TESTES	NUMBER EXAMINED:	8	6	3	3
EPIDIDYMIDES	NUMBER EXAMINED:	8	6	3	3
PROSTATE	NUMBER EXAMINED:	7	6	3	3
SEMINAL VESICLES	NUMBER EXAMINED:	8	6	3	3
COAGULATING GL.	NUMBER EXAMINED:	8	6	3	3
PITUITARY GLAND	NUMBER EXAMINED:	8	6	3	3

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		SEX:	MALE			
		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	8	6	3	3
THYROID GLAND	NUMBER EXAMINED:		8	6	2	2
--M-FOLLICULAR CELL CARCINOMA			1	0	0	0
PARATHYROID GLS.	NUMBER EXAMINED:		6	4	3	2
ADRENAL GLANDS	NUMBER EXAMINED:		8	6	3	2
EYES	NUMBER EXAMINED:		8	6	3	3
HARDERIAN GLANDS	NUMBER EXAMINED:		8	6	3	3
MUSCLE	NUMBER EXAMINED:		8	6	3	3
SKIN	NUMBER EXAMINED:		8	6	2	3
MAMMARY	NUMBER EXAMINED:		0	1	0	0
LYMPH NODE	NUMBER EXAMINED:		2	3	0	0
TAIL	NUMBER EXAMINED:		1	0	1	1
EARS	NUMBER EXAMINED:		1	0	0	1
--M-U/FIBROSARCOMA			1	0	0	0
NOSE/TURBINATES	NUMBER EXAMINED:		1	1	0	1
PREPUTIAL GLAND	NUMBER EXAMINED:		0	0	0	1
BONE	NUMBER EXAMINED:		0	1	0	0
LYMPHO/RET.NEOPL	NUMBER EXAMINED:		1	0	0	0
--M-MALIGNANT LYMPHOMA/LYMPHOCYTIC LEUKEMIA			1	0	0	0
PENIS	NUMBER EXAMINED:		1	0	0	0

** END OF LIST **

T 25C-4
Table 25C (cont.)

T 26A-1
Table 26A
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Incidence of Neoplasms - Female Rats
6 Month Interim Sacrifice

Key

U/ = Unilateral
B- = Benign
SC = Spinal Cord
LN = Lymph Node
GLS = Glands

11 4

T 26A-2
Table 26A (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=B,M,X;SUBSET=I

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
	NUMBER:	10	10	10	10
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	10	0	0	10
CERVICAL SC	NUMBER EXAMINED:	10	0	0	10
THORACIC SC	NUMBER EXAMINED:	10	0	0	10
LUMBAR SC	NUMBER EXAMINED:	10	0	0	10
NERVE	NUMBER EXAMINED:	10	0	0	10
HEART	NUMBER EXAMINED:	10	0	0	10
SALIVARY GLANDS	NUMBER EXAMINED:	10	0	0	10
AORTA	NUMBER EXAMINED:	10	0	0	10
TRACHEA	NUMBER EXAMINED:	10	0	0	10
ESOPHAGUS	NUMBER EXAMINED:	10	0	0	10
LUNGS	NUMBER EXAMINED:	10	9	6	10
MESENTERIC LN	NUMBER EXAMINED:	10	0	6	10
MEDIASTINAL LN	NUMBER EXAMINED:	10	0	0	9
THYMUS	NUMBER EXAMINED:	9	0	0	10
SPLEEN	NUMBER EXAMINED:	10	0	0	10
LIVER	NUMBER EXAMINED:	10	1	0	10
BONE STERNUM	NUMBER EXAMINED:	10	0	0	10
BONE FEMUR	NUMBER EXAMINED:	10	0	0	10
BONE RIB	NUMBER EXAMINED:	8	0	0	9
MARROW STERNUM	NUMBER EXAMINED:	10	0	0	10

*** PATH/TOX SYSTEM OUTPUT ***
A CHRONIC (12-MONTH) ORAL TOXICITY STUDY
OF E2020 IN THE RAT VIA GAVAGE
MICROSCOPIC FINDINGS --- INCIDENCE SUMMARY

PRINTED: 22-OCT-92
PAGE: 3
STUDY NUMBER: 893499

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10
MARROW FEMUR	NUMBER EXAMINED:	10	0	0	10
MARROW RIB	NUMBER EXAMINED:	8	0	0	9
STOMACH	NUMBER EXAMINED:	10	0	1	10
DUODENUM	NUMBER EXAMINED:	10	0	0	10
PANCREAS	NUMBER EXAMINED:	10	0	0	10
JEJUNUM	NUMBER EXAMINED:	10	0	0	10
ILEUM	NUMBER EXAMINED:	10	0	0	10
CECUM	NUMBER EXAMINED:	10	0	0	10
COLON	NUMBER EXAMINED:	10	0	0	10
RECTUM	NUMBER EXAMINED:	10	0	0	10
TONGUE	NUMBER EXAMINED:	10	0	0	9
KIDNEYS	NUMBER EXAMINED:	10	1	0	10
URINARY BLADDER	NUMBER EXAMINED:	10	0	0	9
OVARIES	NUMBER EXAMINED:	10	0	0	10
UTERUS	NUMBER EXAMINED:	10	0	0	10
VAGINA	NUMBER EXAMINED:	10	0	0	9
PITUITARY GLAND	NUMBER EXAMINED:	10	0	0	10
THYROID GLAND	NUMBER EXAMINED:	9	0	0	10
PARATHYROID GLS.	NUMBER EXAMINED:	7	0	0	9

T 26A-4
Table 26A (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10
ADRENAL GLANDS	NUMBER EXAMINED:	9	0	0	10
--B-U/GANGLIONEUROMA		0	0	0	1
EYES	NUMBER EXAMINED:	10	C	0	10
HARDERIAN GLANDS	NUMBER EXAMINED:	10	0	0	10
MUSCLE	NUMBER EXAMINED:	10	0	0	10
SKIN	NUMBER EXAMINED:	10	0	0	10
MAMMARY	NUMBER EXAMINED:	10	0	0	10
--B-FIBROADENOMA		1	0	0	1
LYMPH NODE	NUMBER EXAMINED:	2	0	1	0
TAIL	NUMBER EXAMINED:	1	1	1	1
EARS	NUMBER EXAMINED:	0	1	1	0
EXTREMITY	NUMBER EXAMINED:	0	0	1	0

** END OF LIST **

T 26B-1
Table 26B
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Incidence of Neoplasms - Female Rats
Terminal Sacrifice

Key

B- = Benign
M- = Malignant
SC = Spinal Cord
LN = Lymph Node
GLS = Glands
MESEN/PERITONEUM = Mesentery/Peritoneum
LYMPH/RET. NEOPL. = Lymphoreticular Neoplasm

T 26B-2
Table 26B (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		FEMALE			
GROUP:		-1-	-2-	-3-	-4-
** TOP OF LIST **	NUMBER:	23	23	23	22
BRAIN	NUMBER EXAMINED:	23	0	0	22
CERVICAL SC	NUMBER EXAMINED:	23	0	0	22
THORACIC SC	NUMBER EXAMINED:	23	0	0	22
LUMBAR SC	NUMBER EXAMINED:	23	0	0	22
NERVE	NUMBER EXAMINED:	23	0	0	22
HEART	NUMBER EXAMINED:	23	0	0	22
SALIVARY GLANDS	NUMBER EXAMINED:	23	0	0	22
AORTA	NUMBER EXAMINED:	23	0	0	22
TRACHEA	NUMBER EXAMINED:	23	0	0	22
ESOPHAGUS	NUMBER EXAMINED:	23	0	0	22
LUNGS	NUMBER EXAMINED:	23	2	3	22
MESENTERIC LN	NUMBER EXAMINED:	23	0	1	22
MEDIASTINAL LN	NUMBER EXAMINED:	23	1	0	21
--M-HEMANGIOSARCOMA		0	1	0	0
THYMUS	NUMBER EXAMINED:	22	0	0	21
SPLEEN	NUMBER EXAMINED:	23	0	0	22
LIVER	NUMBER EXAMINED:	23	2	0	22
BONE STERNUM	NUMBER EXAMINED:	23	0	0	21
BONE FEMUR	NUMBER EXAMINED:	23	0	0	22
BONE RIB	NUMBER EXAMINED:	22	0	0	18

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED --- SEX: ------FEMALE------ GROUP: -1-	-2-	-3-	-4-
	NUMBER:	23	23	23	22
MARROW STERNUM	NUMBER EXAMINED:	23	0	0	22
MARROW FEMUR	NUMBER EXAMINED:	23	0	0	22
MARROW RIB	NUMBER EXAMINED:	22	0	0	18
STOMACH	NUMBER EXAMINED:	23	0	1	22
DUODENUM	NUMBER EXAMINED:	23	0	0	22
PANCREAS	NUMBER EXAMINED:	23	0	0	22
JEJUNUM	NUMBER EXAMINED:	23	0	1	22
ILEUM	NUMBER EXAMINED:	23	0	0	22
CECUM	NUMBER EXAMINED:	23	0	0	22
COLON	NUMBER EXAMINED:	23	0	0	22
RECTUM	NUMBER EXAMINED:	23	0	0	22
TONGUE	NUMBER EXAMINED:	23	0	0	22
KIDNEYS	NUMBER EXAMINED:	23	2	0	22
URINARY BLADDER	NUMBER EXAMINED:	23	0	0	22
OVARIES	NUMBER EXAMINED:	23	3	1	21
UTERUS	NUMBER EXAMINED:	23	2	4	22
--B-ENDOMETRIAL STROMAL POLYP		1	0	1	0
VAGINA	NUMBER EXAMINED:	23	0	0	22
PITUITARY GLAND	NUMBER EXAMINED:	23	5	10	22
--B-PARS DISTALIS:ADENOMA		1	2	4	1
THYROID GLAND	NUMBER EXAMINED:	23	1	0	22

T 26B-3
Table 26B (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=B,M,X;SUBSET=I

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		23	23	23	22
PARATHYROID GLS.	NUMBER EXAMINED:	20	0	0	18
ADRENAL GLANDS	NUMBER EXAMINED:	22	2	3	22
EYES	NUMBER EXAMINED:	23	0	0	22
HARDERIAN GLANDS	NUMBER EXAMINED:	23	0	0	22
MUSCLE	NUMBER EXAMINED:	23	0	0	22
SKIN	NUMBER EXAMINED:	22	1	1	22
MAMMARY	NUMBER EXAMINED:	23	2	6	19
--B-FIBROADENOMA		2	1	0	0
--M-CARCINOMA		2	0	0	0
--B-FIBROMA		1	0	0	0
LYMPH NODE	NUMBER EXAMINED:	1	0	0	0
TAIL	NUMBER EXAMINED:	0	0	0	1
EARS	NUMBER EXAMINED:	2	0	0	1
NOSE/TURBINATES	NUMBER EXAMINED:	1	0	0	0
EXTREMITY	NUMBER EXAMINED:	2	0	0	3
LYMPHO/RET.NEOPL	NUMBER EXAMINED:	1	0	0	0
--M-HISTIOCYTIC SARCOMA		1	0	0	0
MESEN/PERITONEUM	NUMBER EXAMINED:	0	1	1	0
PERICARDIUM	NUMBER EXAMINED:	0	1	0	0

** END OF LIST **

T 268-4
Table 268 (cont.)

T 26C-1 89-3499
Table 26C
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

Incidence of Neoplasms - Female Rats
Unscheduled Deaths

Key

B- = Benign
M- = Malignant
SC = Spinal Cord
LN = Lymph Node
GLS = Glands
LYMPH/RET. NEOPL. = Lymphoreticular Neoplasm

TABLE INCLUDES.
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	4
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	2	2	2	4
CERVICAL SC	NUMBER EXAMINED:	2	1	2	4
THORACIC SC	NUMBER EXAMINED:	2	2	2	4
LUMBAR SC	NUMBER EXAMINED:	2	2	2	4
NERVE	NUMBER EXAMINED:	2	2	2	4
HEART	NUMBER EXAMINED:	2	2	2	4
SALIVARY GLANDS	NUMBER EXAMINED:	2	2	2	4
AORTA	NUMBER EXAMINED:	2	2	2	4
TRACHEA	NUMBER EXAMINED:	2	2	2	4
ESOPHAGUS	NUMBER EXAMINED:	2	2	2	4
LUNGS	NUMBER EXAMINED:	2	2	2	4
MESENTERIC LN	NUMBER EXAMINED:	2	2	2	4
MEDIASTINAL LN	NUMBER EXAMINED:	2	2	2	4
THYMUS	NUMBER EXAMINED:	0	2	2	3
SPLEEN	NUMBER EXAMINED:	2	2	2	4
LIVER	NUMBER EXAMINED:	2	2	2	4
BONE STERNUM	NUMBER EXAMINED:	2	2	2	4
BONE FEMUR	NUMBER EXAMINED:	2	2	2	4
BONE RIB	NUMBER EXAMINED:	0	1	2	3
MARROW STERNUM	NUMBER EXAMINED:	2	2	2	4

T 26C-2
Table 26C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=B,N,X;SUBSET=T

--- NUMBER OF ANIMALS AFFECTED ---

ORGAN AND FINDING DESCRIPTION		SEX:	FEMALE			
		GROUP:	-1-	-2-	-3-	-4-
	NUMBER:		2	2	2	4
MARROW FEMUR	NUMBER EXAMINED:		2	2	2	4
MARROW RIB	NUMBER EXAMINED:		0	1	2	3
STOMACH	NUMBER EXAMINED:		2	2	2	4
DUODENUM	NUMBER EXAMINED:		2	2	2	4
PANCREAS	NUMBER EXAMINED:		2	2	2	4
JEJUNUM	NUMBER EXAMINED:		2	1	2	4
ILEUM	NUMBER EXAMINED:		2	1	2	4
CECUM	NUMBER EXAMINED:		2	1	2	4
COLON	NUMBER EXAMINED:		2	2	2	4
RECTUM	NUMBER EXAMINED:		2	2	2	4
TONGUE	NUMBER EXAMINED:		2	2	2	4
KIDNEYS	NUMBER EXAMINED:		2	2	2	4
URINARY BLADDER	NUMBER EXAMINED:		2	2	2	4
OVARIES	NUMBER EXAMINED:		2	2	2	4
UTERUS	NUMBER EXAMINED:		2	2	2	4
VAGINA	NUMBER EXAMINED:		2	2	2	4
PITUITARY GLAND	NUMBER EXAMINED:		1	1	2	4
--B-PARS DISTALIS:ADENOMA			0	1	0	0
THYROID GLAND	NUMBER EXAMINED:		2	1	2	4
--B-C-CELL ADENOMA			0	0	0	1
PARATHYROID GLS	NUMBER EXAMINED:		0	1	2	3

T 26C-3
Table 26C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=B,M,X;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
	NUMBER:	2	2	2	4
ADRENAL GLANDS	NUMBER EXAMINED:	2	2	2	4
EYES	NUMBER EXAMINED:	2	2	2	4
HARDERIAN GLANDS	NUMBER EXAMINED:	2	2	2	4
MUSCLE	NUMBER EXAMINED:	2	2	2	4
SKIN	NUMBER EXAMINED:	2	2	2	4
--M-BASAL CELL CARCINOMA		1	0	0	0
--M-FIBROSARCOMA		0	0	0	1
MAMMARY	NUMBER EXAMINED:	2	2	2	4
LYMPH NODE	NUMBER EXAMINED:	1	1	1	0
EARS	NUMBER EXAMINED:	0	1	0	0
BONE	NUMBER EXAMINED:	0	0	1	0
LYMPHO/RET NEOPL	NUMBER EXAMINED:	1	0	1	0
--M-MALIGNANT LYMPHOMA/LYMPHOCYTIC LEUKEMIA		1	0	0	0
--M-GRANULOCYTIC LEUKEMIA		0	0	1	0
MEDIASTINUM	NUMBER EXAMINED:	0	0	1	0
PERICARDIUM	NUMBER EXAMINED:	0	0	0	1
DIAPHRAGM	NUMBER EXAMINED:	0	0	0	1

** END OF LIST **

T 26C-4
Table 26C (cont.)

T 28A-1
Table 28A
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Non-Neoplastic Lesion Incidence - Female Rats
6 Month Interim Sacrifice

Key

U/ = Unilateral
B/ = Bilateral
SC = Spinal Cord
LN = Lymph Node
GLS = Glands

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		FEMALE			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	10	0	0	10
CERVICAL SC	NUMBER EXAMINED:	10	0	0	10
THORACIC SC	NUMBER EXAMINED:	10	0	0	10
LUMBAR SC	NUMBER EXAMINED:	10	0	0	10
NERVE	NUMBER EXAMINED:	10	0	0	10
HEART	NUMBER EXAMINED:	10	0	0	10
--MYOCARDIUM:HEMORRHAGE		1	0	0	0
SALIVARY GLANDS	NUMBER EXAMINED:	10	0	0	10
AORTA	NUMBER EXAMINED:	10	0	0	10
TRACHEA	NUMBER EXAMINED:	10	0	0	10
ESOPHAGUS	NUMBER EXAMINED:	10	0	0	10
LUNGS	NUMBER EXAMINED:	10	9	6	10
--CHRONIC INTERSTITIAL PNEUMONIA		8	8	5	9
--CONGESTION		1	0	0	0
--HEMORRHAGE		1	0	1	0
--EMPHYSEMA		6	8	3	6
MESENTERIC LN	NUMBER EXAMINED:	10	0	0	10
MEDIASTINAL LN	NUMBER EXAMINED:	10	0	0	9
THYMUS	NUMBER EXAMINED:	9	0	0	10
--CYST(S)		3	0	0	3
--LYMPHOID CELL ATROPHY		1	0	0	0
SPLEEN	NUMBER EXAMINED:	10	0	0	10
LIVER	NUMBER EXAMINED:	10	1	0	10

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

		--- NUMBER-OF-ANIMALS-AFFECTED ---			
	SEX:	------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	10	10	10	10
BONE STERNUM	NUMBER EXAMINED:	10	0	0	10
BONE FEMUR	NUMBER EXAMINED:	10	0	0	10
BONE RIB	NUMBER EXAMINED:	8	0	0	9
MARROW STERNUM	NUMBER EXAMINED:	10	0	0	10
MARROW FEMUR	NUMBER EXAMINED:	10	0	0	10
MARROW RIB	NUMBER EXAMINED:	8	0	0	9
STOMACH	NUMBER EXAMINED:	10	0	1	10
DUODENUM	NUMBER EXAMINED:	10	0	0	10
PANCREAS	NUMBER EXAMINED:	10	0	0	10
JEJUNUM	NUMBER EXAMINED:	10	0	0	10
ILEUM	NUMBER EXAMINED:	10	0	0	10
CECUM	NUMBER EXAMINED:	10	0	0	10
--LUMEN:NEMATODES		1	0	0	0
COLON	NUMBER EXAMINED:	10	0	0	10
RECTUM	NUMBER EXAMINED:	10	0	0	10
TONGUE	NUMBER EXAMINED:	10	0	0	9
KIDNEYS	NUMBER EXAMINED:	10	1	0	10
--A/CHRONIC NEPHROPATHY		0	0	0	1
--B/CHRONIC NEPHROPATHY		3	0	0	2
--B/CORTICAL CYST(S)		0	0	0	1
--B/TUBULAR MINERALIZATION		2	0	0	0
URINARY BLADDER	NUMBER EXAMINED:	10	0	0	9

TABLE INCLUDES
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		10	10	10	10
OVARIES	NUMBER EXAMINED:	10	0	0	10
UTERUS	NUMBER EXAMINED:	10	0	0	10
--HYDRONETRA		0	0	0	1
VAGINA	NUMBER EXAMINED:	10	0	0	9
PITUITARY GLAND	NUMBER EXAMINED:	10	0	0	10
--CYST(S)		1	0	0	0
--PARS DISTALIS HYPERPLASIA/HYPERTROPHY		1	0	0	0
THYROID GLAND	NUMBER EXAMINED:	9	0	0	10
--THYROGLOSSAL CYST(S)		0	0	0	1
PARATHYROID GLS	NUMBER EXAMINED:	7	0	0	9
ADRENAL GLANDS	NUMBER EXAMINED:	9	0	0	10
--B/CORTEX:VACUOLATION		1	0	0	0
EYES	NUMBER EXAMINED:	10	0	0	10
--B/RETROBULBAR HEMORRHAGE		2	0	0	2
HARDERIAN GLANDS	NUMBER EXAMINED:	10	0	0	10
MUSCLE	NUMBER EXAMINED:	10	0	0	10
SKIN	NUMBER EXAMINED:	10	0	0	10
MAMMARY	NUMBER EXAMINED:	10	0	0	10
LYMPH NODE	NUMBER EXAMINED:	2	0	1	0
--CONGESTION/HEMORRHAGE		2	0	1	0
--PLASMA CELL HYPERPLASIA		1	0	0	0
TAIL	NUMBER EXAMINED:	1	1	1	1
--HYPERKERATOSIS		1	1	1	1
EARS	NUMBER EXAMINED:	0	1	1	0
--B/CHRONIC ACTIVE INFLAMMATION		0	1	1	0

** CONTINUED ON NEXT PAGE **

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                                                                PRINTED: 22-OCT-92
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*** PATHOLOGY SYSTEM OUTPUT ***
A CHRONIC (12-MONTH) ORAL TOXICITY STUDY                        STUDY NUMBER: 893499
   OF E2020 IN THE RAT VIA GAVAGE
MICROSCOPIC FINDINGS --- INCIDENCE SUMMARY

---------------------------------------------------------------------------
                                                  ---N U M B E R - O F - A N I M A L S - A F F E C T E D---

TABLE INCLUDES:                                        SEX:  -----MALE-----   -----FEMALE-----
   SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL                GROUP:  -1-   -2-   -3-   -4-
   DEATH=1;FIND=P;SUBSET=T
                                                       NUMBER:  10    10    10    10
ORGAN AND FINDING DESCRIPTION                          -----  ----  ----  ----  ----
---------------------------------------------------------------------------
** FROM PREVIOUS PAGE **
EARS                          NUMBER EXAMINED:          0     1     1     0
   --B/CORNIFIED CELL HYPERPLASIA                       0     1     1     0

EXTREMITY                     NUMBER EXAMINED:          0     0     1     0
   --CHRONIC ACTIVE INFLAMMATION                        0     0     1     0
** END OF LIST **
```


11 44

T 288-1
Table 288
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Non-Neoplastic Lesion Incidence - Female Rats
Terminal Sacrifice

Key

U/ = Unilateral
B/ = Bilateral
SC = Spinal cord
LN = Lymph Node
GLS = Glands
MESEN/PERITONEUM = Mesentery/Peritoneum
LYMPH/RET. NEOPL. = Lymphoreticular Neoplasm

T 288-2
Table 288 (cont.)

TABLE INCLUDES
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		SEX:	FEMALE			
		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	23	23	23	22
** TOP OF LIST **						
BRAIN	NUMBER EXAMINED:		23	0	0	22
CERVICAL SC	NUMBER EXAMINED:		23	0	0	22
THORACIC SC	NUMBER EXAMINED:		23	0	0	22
LUMBAR SC	NUMBER EXAMINED:		23	0	0	22
NERVE	NUMBER EXAMINED:		23	0	0	22
HEART	NUMBER EXAMINED:		23	0	0	22
--CHRONIC MYOCARDITIS/FIBROSIS			0	0	0	1
SALIVARY GLANDS	NUMBER EXAMINED:		23	0	0	22
AORTA	NUMBER EXAMINED:		23	0	0	22
TRACHEA	NUMBER EXAMINED:		23	0	0	22
ESOPHAGUS	NUMBER EXAMINED:		23	0	0	22
LUNGS	NUMBER EXAMINED:		23	2	3	22
--CHRONIC INTERSTITIAL PNEUMONIA			2	1	1	4
--HEMORRHAGE			1	0	0	0
--EMPHYSEMA			4	1	1	7
--ALVEOLAR MACROPHAGES:ACCUMULATION			0	1	0	0
--ATELECTASIS			0	0	0	1
--ACUTE BRONCHOPNEUMONIA			0	0	0	1
--CHRONIC PLEURITIS/ADHESIONS			0	0	0	1
MESENTERIC LN	NUMBER EXAMINED:		23	0	1	22
MEDIASTINAL LN	NUMBER EXAMINED:		23	1	0	21
--RETICULOENDOTHELIAL CELL HYPERPLASIA			0	0	0	1
THYMUS	NUMBER EXAMINED:		22	0	0	21
--CYST(S)			6	0	0	6
--LYMPHOID CELL ATROPHY			0	0	0	1

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=I

ORGAN AND FINDING DESCRIPTION	NUMBER	SEX: FEMALE -1-	-2-	-3-	-4-
		--- NUMBER OF ANIMALS AFFECTED ---			
SPLEEN	NUMBER EXAMINED:	23	0	0	22
--RETICULOENDOTHELIAL CELLS: BROWN PIGMENT		1	0	0	1
--EXTRAMEDULLARY HEMATOPOIESIS		1	0	0	2
LIVER	NUMBER EXAMINED:	23	2	0	22
--HEPATOCELLULAR VACUOLATION		2	0	0	1
--TENSION LIPIDOSIS		2	0	0	0
--COAGULATIVE NECROSIS		0	0	0	1
--ACUTE INFLAMMATION		0	0	0	1
--PELIOSIS HEPATIS		1	0	0	0
BONE STERNUM	NUMBER EXAMINED:	23	0	0	21
BONE FEMUR	NUMBER EXAMINED:	23	0	0	22
BONE RIB	NUMBER EXAMINED:	22	0	0	18
MARROW STERNUM	NUMBER EXAMINED:	23	0	0	22
MARROW FEMUR	NUMBER EXAMINED:	23	0	0	22
MARROW RIB	NUMBER EXAMINED:	22	0	0	18
STOMACH	NUMBER EXAMINED:	23	0	1	22
DUODENUM	NUMBER EXAMINED:	23	0	0	22
PANCREAS	NUMBER EXAMINED:	23	0	0	22
--ACINAR ATROPHY		1	0	0	1
JEJUNUM	NUMBER EXAMINED:	23	0	1	22
ILEUM	NUMBER EXAMINED:	23	0	0	22
CECUM	NUMBER EXAMINED:	23	0	0	22
--LUMEN:NEMATODES		1	0	0	0
COLON	NUMBER EXAMINED:	23	0	0	22
--LUMEN:NEMATODES		1	0	0	2

*** PATH/TOX SYSTEM OUTPUT ***
A CHRONIC (12-MONTH) ORAL TOXICITY STUDY
OF C2020 IN THE RAT VIA GAVAGE
MICROSCOPIC FINDINGS --- INCIDENCE SUMMARY

PRINTED: 22-OCT-92
PAGE: 4

STUDY NUMBER: 893499

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		FEMALE			
GROUP:		-1-	-2-	-3-	-A-
NUMBER:		23	23	23	22
RECTUM	NUMBER EXAMINED:	23	0	0	22
--LUMEN:NEMATODES		3	0	0	2
TONGUE	NUMBER EXAMINED:	23	0	0	22
KIDNEYS	NUMBER EXAMINED:	23	2	0	22
--B/CHRONIC NEPHROPATHY		10	2	0	0
--U/HYDRONEPHROSIS		0	1	0	1
--U/CORTICAL CYST(S)		1	0	0	0
--B/TUBULAR MINERALIZATION		3	0	0	3
--U/PELVIS:UROLITH(S)		0	0	0	1
--B/TUBULAR EPITHELIUM:CYTOPLASMIC ACIDOPHILIC DROPLETS		1	0	0	1
URINARY BLADDER	NUMBER EXAMINED:	23	0	0	22
OVARIES	NUMBER EXAMINED:	23	3	1	21
--CYST(S)		2	1	0	0
--B/CYSTS		0	1	1	0
UTERUS	NUMBER EXAMINED:	23	2	4	22
--ATROPHY		0	1	1	2
--ENDOMETRIAL CYST(S)		2	1	2	2
VAGINA	NUMBER EXAMINED:	23	0	0	22
PITUITARY GLAND	NUMBER EXAMINED:	23	5	10	22
--CYST(S)		0	1	1	1
--PARS DISTALIS:HYPERPLASIA/HYPERTROPHY		0	0	1	1
--HEMORRHAGE		7	1	6	2
THYROID GLAND	NUMBER EXAMINED:	23	1	0	22
--THYROGLOSSAL CYST(S)		2	0	0	0
PARATHYROID GLS.	NUMBER EXAMINED:	20	0	0	18
--CYST(S)		1	0	0	0
ADRENAL GLANDS	NUMBER EXAMINED:	22	2	3	22
--B/CORTEX:VACUOLATION		1	0	0	0
--B/CONGESTION		7	1	2	6

** CONTINUED ON NEXT PAGE **

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		23	23	23	22
** FROM PREVIOUS PAGE **					
ADRENAL GLANDS	NUMBER EXAMINED:	22	2	3	22
--U/HEMORRHAGE/HEMATOCYSTS		0	0	0	1
--B/HEMORRHAGE/HEMATOCYSTS		3	1	1	5
--U/FOCUS/AREA OF CELL ALTERATION/DEGENERATION		2	1	0	3
EYES	NUMBER EXAMINED:	23	0	0	22
--U/RETROBULBAR HEMORRHAGE		6	0	0	8
--U/PHTHISIS BULBI		0	0	0	1
HARDERIAN GLANDS	NUMBER EXAMINED:	23	0	0	22
--U/ACUTE INFLAMMATION		1	0	0	0
MUSCLE	NUMBER EXAMINED:	23	0	0	22
SKIN	NUMBER EXAMINED:	22	1	1	22
--ACUTE INFLAMMATION/NECROSIS		0	1	0	0
--HYPOTRICHOSIS		1	0	0	0
--SUBACUTE/CHRONIC INFLAMMATION		0	0	0	1
MAMMARY	NUMBER EXAMINED:	23	2	6	19
--GALACTOCELES		10	1	6	7
--ACINAR HYPERPLASIA		0	0	0	1
--ACUTE INFLAMMATION/NECROSIS		1	0	0	0
LYMPH NODE	NUMBER EXAMINED:	1	0	0	0
--PLASMA CELL HYPERPLASIA		1	0	0	0
TAIL	NUMBER EXAMINED:	0	0	0	1
--HYPERKERATOSIS		0	0	0	1
EARS	NUMBER EXAMINED:	2	0	0	1
--B/CHRONIC ACTIVE INFLAMMATION		2	0	0	1
--B/CHONDROID CELL HYPERPLASIA		2	0	0	1
NOSE/TURBINATES	NUMBER EXAMINED:	1	0	0	0
--ACUTE INFLAMMATION/NECROSIS		1	0	0	0
EXTREMITY	NUMBER EXAMINED:	2	0	0	3
--CHRONIC ACTIVE INFLAMMATION		1	0	0	1

** CONTINUED ON NEXT PAGE **

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		23	23	23	22
** FROM PREVIOUS PAGE **					
EXTREMITY	NUMBER EXAMINED:	2	0	0	3
--OSSEOUS HYPERPLASIA		1	0	0	0
--ACUTE INFLAMMATION/NECROSIS		0	0	0	2
LYMPHO/RET.NEOPL	NUMBER EXAMINED:	1	0	0	0
MESEN/PERITONEUM	NUMBER EXAMINED:	0	1	1	0
--FAT NECROSIS		0	1	1	0
PERICARDIUM	NUMBER EXAMINED:	0	1	0	0
** END OF LIST **					

T 28C-1
Table 28C
A Chronic (12-Month) Oral Toxicity Study
of E2020 in the Rat via Gavage

89-3499

Non-Neoplastic Lesion Incidence - Female Rats
Unscheduled Deaths

Key

U/ = Unilateral
B/ = Bilateral
SC = Spinal Cord
LN = Lymph Node
GLS = Glands
LYMPH/RET. NEOPL. = Lymphoreticular Neoplasm

11 fff

*** PATHOTOX SYSTEM OUTPUT ***
A CHRONIC (12-MONTH) ORAL TOXICITY STUDY
OF E2020 IN THE RAT VIA GAVAGE
MICROSCOPIC FINDINGS --- INCIDENCE SUMMARY

PRINTED: 22-OCT-92
PAGE: 2

STUDY NUMBER: 893499

--- N U M B E R - O F - A N I M A L S - A F F E C T E D ---

TABLE INCLUDES SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL DEATH=A,S,D,O;FIND=P;SUBSET=T	SEX:	FEMALE			
	GROUP:	1	2	3	4
ORGAN AND FINDING DESCRIPTION	NUMBER:	2	2	2	4
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	2	2	2	4
--COMPRESSION BY PITUITARY NEOPLASM		0	1	0	0
CERVICAL SC	NUMBER EXAMINED:	2	1	2	4
THORACIC SC	NUMBER EXAMINED:	2	2	2	4
LUMBAR SC	NUMBER EXAMINED:	2	2	2	4
NERVE	NUMBER EXAMINED:	2	2	2	4
HEART	NUMBER EXAMINED:	2	2	2	4
SALIVARY GLANDS	NUMBER EXAMINED:	2	2	2	4
AORTA	NUMBER EXAMINED:	2	2	2	4
TRACHEA	NUMBER EXAMINED:	2	2	2	4
--ACUTE/SUBACUTE INFLAMMATION		0	1	0	0
ESOPHAGUS	NUMBER EXAMINED:	2	2	2	4
--ACUTE/SUBACUTE INFLAMMATION		0	1	0	1
LUNGS	NUMBER EXAMINED:	2	2	2	4
--CHRONIC INTERSTITIAL PNEUMONIA		0	0	1	3
--CONGESTION		0	2	1	4
--HEMORRHAGE		0	1	1	1
--EDEMA		0	1	0	0
--EMPHYSEMA		0	0	0	3
--ACUTE PERIVASCULITIS/ADHESIONS		0	0	1	0
--ALVEOLAR MACROPHAGES: ACCUMULATION		0	0	0	1
--GRANULOMA(S)/GRANULOMATOUS INFLAMMATION		1	0	0	0
MESENTERIC LN	NUMBER EXAMINED:	2	2	2	4
MEDIASTINAL LN	NUMBER EXAMINED:	2	2	2	4
--CONGESTION/HEMORRHAGE		0	1	2	0
THYMUS	NUMBER EXAMINED:	0	2	2	3

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11 995

T 28C-3
Table 28C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A.S.D.O;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
	NUMBER:	2	2	2	4
SPLEEN	NUMBER EXAMINED:	2	2	2	4
--EXTRAMEDULLARY HEMATOPOIESIS		1	0	0	1
LIVER	NUMBER EXAMINED:	2	2	2	4
--CONGESTION		0	2	1	4
BONE STERNUM	NUMBER EXAMINED:	2	2	2	4
BONE FEMUR	NUMBER EXAMINED:	2	2	2	4
BONE RIB	NUMBER EXAMINED:	0	1	2	3
MARROW STERNUM	NUMBER EXAMINED:	2	2	2	4
MARROW FEMUR	NUMBER EXAMINED:	2	2	2	4
MARROW RIB	NUMBER EXAMINED:	0	1	2	3
STOMACH	NUMBER EXAMINED:	2	2	2	4
--FORESTOMACH:SQUAMOUS CELL HYPERPLASIA		0	1	0	0
--GLANDULAR MUCOSA:ACUTE INFLAMMATION/NECROSIS		1	0	0	0
DUODENUM	NUMBER EXAMINED:	2	2	2	4
PANCREAS	NUMBER EXAMINED:	2	2	2	4
JEJUNUM	NUMBER EXAMINED:	2	1	2	4
ILEUM	NUMBER EXAMINED:	2	1	2	4
CECUM	NUMBER EXAMINED:	2	1	2	4
COLON	NUMBER EXAMINED:	2	2	2	4
--LUMEN:NEMATODES		0	0	0	1
RECTUM	NUMBER EXAMINED:	2	2	2	4
--LUMEN:NEMATODES		1	0	0	0
TONGUE	NUMBER EXAMINED:	2	2	2	4

T 28C-4
Table 28C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,0;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	4
KIDNEYS	NUMBER EXAMINED:	2	2	2	4
--B/CHRONIC NEPHROPATHY		0	1	0	1
--B/CONGESTION		0	2	1	3
--B/TUBULAR EPITHELIUM:CYTOPLASMIC ACIDOPHILIC DROPLETS		1	0	0	1
URINARY BLADDER	NUMBER EXAMINED:	2	2	2	4
--ACUTE/SUBACUTE INFLAMMATION		1	0	0	0
--UROTHELIAL HYPERPLASIA		1	0	0	0
OVARIES	NUMBER EXAMINED:	2	2	2	4
--B/CONGESTION		0	0	1	1
UTERUS	NUMBER EXAMINED:	2	2	2	4
--HYDROMETRA		0	0	2	1
VAGINA	NUMBER EXAMINED:	2	2	2	4
--CYST(S)		1	0	0	0
PITUITARY GLAND	NUMBER EXAMINED:	1	1	2	4
--PARS DISTALIS:HYPERPLASIA/HYPERTROPHY		1	0	0	0
--HEMORRHAGE		1	0	0	0
THYROID GLAND	NUMBER EXAMINED:	2	1	2	4
PARATHYROID GLS.	NUMBER EXAMINED:	0	1	2	3
ADRENAL GLANDS	NUMBER EXAMINED:	2	2	2	4
--B/CONGESTION		1	2	0	0
--B/ACCESSORY ADRENAL TISSUE		0	1	0	0
--B/HEMORRHAGE/HEMATOCYSTS		0	0	0	1
EYES	NUMBER EXAMINED:	2	2	2	4
--B/ACUTE KERATITIS/NEOVASCULARIZATION		0	0	0	1
HARDERIAN GLANDS	NUMBER EXAMINED:	2	2	2	4
MUSCLE	NUMBER EXAMINED:	2	2	2	4

*** PATH/TOX SYSTEM OUTPUT ***
A CHRONIC (12-MONTH) ORAL TOXICITY STUDY
OF E2020 IN THE RAT VIA GAVAGE
MICROSCOPIC FINDINGS --- INCIDENCE SUMMARY

STUDY NUMBER: 893499

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=A,S,D,O;FIND=P;SUBSET=T

--- NUMBER-OF-ANIMALS-AFFECTED ---

ORGAN AND FINDING DESCRIPTION		SEX:	FEMALE			
		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	2	2	2	4
SKIN	NUMBER EXAMINED:		2	2	2	4
--ACUTE INFLAMMATION/NECROSIS			1	0	0	1
MAMMARY	NUMBER EXAMINED:		2	2	2	4
--GALACTOCELES			1	0	0	0
LYMPH NODE	NUMBER EXAMINED:		1	1	1	0
--LYMPHOID CELL HYPERPLASIA			0	0	1	0
--PLASMA CELL HYPERPLASIA			1	0	0	0
EARS	NUMBER EXAMINED:		0	1	0	0
--M/CHONDROID CELL HYPERPLASIA			0	1	0	0
BONE	NUMBER EXAMINED:		0	0	1	0
LYMPHO/RET.NEOPL	NUMBER EXAMINED:		1	0	1	0
MEDIASTINUM	NUMBER EXAMINED:		0	0	1	0
--ACUTE INFLAMMATION			0	0	1	0
--FOREIGN MATERIAL			0	0	1	0
PERICARDIUM	NUMBER EXAMINED:		0	0	0	1
DIAPHRAGM	NUMBER EXAMINED:		0	0	0	1

** END OF LIST **

T 28C-5
Table 28C (cont.)

3 MONTH P.O. TOXICITY IN DOGS

Beagle dogs were originally scheduled to receive doses of 0, 0.3, 1, 3, and 8 mg/kg/day (in capsules); however, due to deaths at HD during the first 2 days, animals were reassigned to the following doses. (Underlined dogs are those survivors at 8 mg/kg which were reassigned. Also as indicated, 2 controls and 4 at 3 mg/kg were kept for a 5 week recovery period):

Dose group			Sex	Animal No.				
Control			Male	7153	7154	7159	7160*	
			Female	7648	7653	7657	7655*	
E2020	0.3	mg/kg	Male	7149	7156	7161		
			Female	7649	7661	7665		
E2020	1	mg/kg	Male	7147	7155	7158		
			Female	7645	7646	7658		
E2020	3	mg/kg	Male	7150	7152	7157	7148*	7151*
			Female	7656	7659	7662	7650*	7663*
E2020	6	mg/kg	Male	7145				

*; Animals scheduled to be maintained undosed for 5 weeks after completion of the treatment period for recovery testing.

Originally, 10 dogs were to have received 8 mg/kg; of these 1 died 7 hours after the first dose and 4 died (including 1 F sacrificed in extremis) 3-24 hours after the second dose. Signs prior to death included salivation, fasciculation, tremors, and convulsions. Blood chemistry was done in the F which was sacrificed and showed elevated ALT, AST, LDH, CPK, AP, LAP,GGTP, BUN, and total bilirubin, and low glucose. Postmortem exam in these dogs showed, grossly and microscopically, extensive subendocardial hemorrhage in the left ventricle and, microscopically, myofibrillar degeneration in left ventricle wall, papillary muscle, and septum; it was stated that the lesions were multifocal and showed very slight eosinophilia of the myocardial fibers. Similar

changes were seen in dogs which died in an acute toxicity study; as discussed in the "Acute Toxicity" section (above) they were considered to be secondary effects of lethal, hypoxic doses of the drug.

There were no drug-related clinical signs at 0.3 and 1 mg/kg. Fasciculation and tremors were seen at 3 and 6 mg/kg; these were mainly seen in the first 2 weeks. Mucous stools were seen with increased frequency at 3 mg/kg. No drug-related signs were seen during the recovery period.

There were no drug effects on periodic ophthalmoscopic exams, EKG (note that the time after dosing when measured not given; thus it is not clear if EKG was measured near time of peak blood levels), hematology, blood chemistry (including pseudo-cholinesterase) or (24 hour) urinalysis. (One dog in the 8-3 mg/kg group had elevated ALT at week 1 but not at later times; no liver histopathology seen in this dog). There were no clear drug effects on gross or microscopic organ exam. (Submaxillary gland weights were equivocally increased at 3-6 mg/kg, without histological correlate). Overall, 1 mg/kg was a no-effect dose in this study, 3-6 mg/kg produced cholinergic signs, and 8 mg/kg produced pronounced cholinergic signs and deaths. (Note that only 1 dog was studied at 6 mg/kg).

Plasma levels of unchanged drug measured at various times were generally below the level of detection (1 ng/ml) at the lowest dose. Results of the 1 and 3 mg/kg dose are shown in the attached tables. At 1 mg/kg , C_{max} increased over the study, suggesting continuing accumulation, although the data were rather limited due to the low levels seen. At 3 mg/kg, levels increased between days 1 and 35 but not between days 35 and 91. Cmax values at 3 mg/kg were about 15-20x those at 1 mg/kg. Limited data were obtained for the 8 mg/kg group due to early deaths and re-assignment of survivors to lower dose groups; Cmax values obtained in 6 of these dogs on the first day of dosing ranged from 305 to 431 ng/ml. (Of these the values in the 4 that died were not clearly greater than those in the 2 survivors). Comparing these values with the day 1 C_{max} values for the 3 mg/kg group (see table) indicates a greater than proportional increase with dose between the 3 and 8 mg/kg doses (i.e., approximately 7x increase in Cmax).

In this study, liver samples were obtained at termination from 2/sex at 0, 0.3, 1, and 3 mg/kg, and 1 M at 6 mg/kg, and analyzed for cytochromes b5 and P 450, and various drug metabolizing enzymes. No drug effects were seen.

Table 2: Pharmacokinetic Parameters During 13-weeks Administration of E2020 to Beagle Dogs at Single, Daily, Oral Doses of 1 mg/kg

Study Day	C_{max} (ng/ml)	C_{min} (ng/ml)	t_{max} (hr)
1st	4.0±1.4	NS	1.0-4.0
8th	NM	ND	NM
35th	6.6±2.0	ND	1.0-2.0
91st	8.9±2.6	ND	1.0-2.0

n = 5 (1 female excluded, due to abnormally high values on 1st day)
ND: not detected (< 1 ng/ml, as 1.0 ml volumes of plasma were analyzed), C_{min} was also ND on the 4th day.
NM: not measured
NS: no sample collected 24 hours after the first dose.

Table 1: Pharmacokinetic Parameters During 13-weeks Administration of E2020 to Beagle Dogs at Single, Daily, Oral Doses of 3 mg/kg.

Study Day	C_{max} (ng/ml)	C_{min} (ng/ml)	t_{max} (hr)	$AUC_{0\text{-}8hr}$ (ng•hr/ml)
1st	54 ± 12	ND	1.0-2.0	140 ±35
8th	NM	6.2±2.2	NM	NM
35th	154±18	8.3±4.3	1.0-1.5	598±107
91st	153±26	8.6±3.7	1.0-1.5	550±108

n = 6 (3 males + 3 females)
ND: not detected (< 2 ng/ml, as 0.5 ml volumes of plasma were analyzed) 24 hours after the first dose administration, C_{min} was also ND on the 4th day.
NM: not measured

ONE YEAR P.O. TOXICITY IN DOGS:

A) Dosage

6/sex at 0, 0.6, 2.0, or 5.0 mg/kg/day, in capsules.

2/sex/group were sacrificed at 6 months. (See attached table which also indicates times of lab tests).

Strain: beagle
Drug Lot #s: 88020201, 89120401

B) Results

1) Observed signs

a) Salivation - all doses

b) Tremors, hyperactivity, and lacrimation seen at HD and (in only a very few instances) at MD and (hyperactivity only) LD.

c) Incidence of "swollen mammaries" increased in all F groups; not D-R and not considered treatment-related by the sponsor.

2) Mortality

One control F died day 11.

3) Bodyweights

Weights in HD F were slightly (generally < 10%) below respective controls throughout most of the treatment period; the report states that this was not statistically significant and not considered drug-related.

4) Food consumption

Values were expressed as grams consumed per kg bodyweight thus making evaluation of food consumption per se difficult. The only clear drug effect was a decrease at HD of both sexes week 1 (g/kg consumption ~ 2/3 of control).

5) Water consumption

(Measured pre - Tx and months 3, 6, 9, and 12, over a 7 day period).

As above, results are expressed on a per kg bodyweight basis, making evaluation of the data difficult. The study report concluded there was a decrease in

HD M since values were 55-60% of control; however values were also lower pre-Tx, although to a smaller degree (79%).

6) Ophthalmoscopy

(Done pre-Tx and at months 3, 6, 12)

No drug effects

7) Hematology

(Done pre-Tx and at months 3, 6, 9, 12)

No drug effects.

Parameters measured: RBC, Hb, Hct, platelets, reticulocytes, WBC, differential, RBC morphology, PT, APTT.

8) Blood chemistry

(Done pre-Tx and at months 3, 6, 9, 12)

No clear drug effects.

One HD M had elevated AST and ALT at 3 months and elevated ALT at 9 months. Another HD M and an MD M had elevated ALT at 9 months. An HD F had elevated ALT at 12 months. These elevations were 3-5x the highest concurrent control. The affected animals did not have liver histopathology different from that seen scattered across all groups in the study.

Other parameters measured: AP, total bilirubin, LDH, CPK, glucose, cholesterol, triglycerides, phospholipids, total protein, albumin, protein electrophoresis, BUN, creatinine, uric acid, Na, K, Cl, Ca, phosphorous.

9) Urinalysis

(Done pre-Tx and at months 3, 6, 9, 12)

Decreases in 24 hour urine volume and electrolyte (Na, K, Cl) excretion decreased in MD and HD (and occasionally LD) M, and in HD (and occasionally MD) F, at most times. Magnitude of effects were generally D-R. Magnitude of effects did not clearly increase over time with the exception that in M, effects tended to be less at 3 months than at later times. Mean values at HD were generally 1/3 - 2/3 control means. There was no clear drug effect on urinary electrolyte concentrations.

10) Plasma levels of parent drug

Samples taken from 3/sex at 1, 2, 4, 8, and 24 hours post-dosing on days 1, 28, 182, 364. Results shown in sponsor's summary table, attached. Levels increased greater than proportionally to dose. Levels increased with increasing duration of treatment, particularly between days 1 and 28 (2-4x). There were no apparent sex differences (not shown in table).

11) Organ weights

No clear drug effects. Submaxillary gland weights were equivocally slightly decreased in all M groups at termination, not D-R.

12) Gross pathology

No summary table presented. The report implies no drug effects .

13) Microscopic pathology

(Routine exam done in all animals).

No clear drug effects. A few findings noted by the sponsor to be of increased incidence in the treated groups, but not considered to be drug-related based on low severity and on historical incidence, are shown in the attached table. These equivocal increases were seen only in one sex each, except for thymic cysts which were said to be embryologic remnants. The complete histopathology tables are also attached.

3. METHODS (cont.):

E. Experimental Outline:

Group	Test Substance	Dose[a] Level (mg/kg/day)	Number of Animals: Initial Number on Test M	F	Pharmacokinetic[b] Study M	F	Clinical[c] Laboratory Studies: Pre M	F	Mos 3&6 M	F	Mos 9&12 M	F	Necropsy: Mo 6 M	F	Mo 12 M	F	Histopathology[d]: Mo 6 M	F	Mo 12 M	F
I (control)	Vehicle	0[e]	6	6[f]	3	3	6	6	6	5[f]	4	3[f]	2	2	4	3[f]	2	3[f]	4	3
II (low-)	E2020	0.6	6	6	3	3	6	6	6	6	4	4	2	2	4	4	2	2	4	4
III (mid)	E2020	2.0	6	6	3	3	6	6	6	6	4	4	2	2	4	4	2	2	4	4
IV (high-)	E2020	5.0	6	6	3	3	6	6	6	6	4	4	2	2	4	4	2	2	4	4

[a]Dosing was based on =100% activity of the (technical or formulated) grade of the test substance as supplied by the sponsor.

[b]For pharmacokinetic sampling, three animals/sex/group were sampled before compound administration and at 1, 2, 4, 8 and 24 hours after dosing. Blood was drawn on Day 1 and at 1, 6 and 12 months of dosing. One ml of plasma was collected from each dog at each time point. The same animals were sampled throughout the study. The animals were assigned to this group at the beginning of the study.

[c]Biochemistry, hematology and urinalysis were conducted pretest and at Months 3, 6, 9 and 12.

[d]Histopathology was performed on all animals sacrificed at the Month 6 Interim, Month 12 Terminal Sacrifice and any animals dying spontaneously prior to the scheduled sacrifices.

[e]Control animals received gelatin capsules containing 5 mg/kg/day of the carrier (88:2 mixture of glactose:hydroxypropyl cellulose).

[f]One animal in Group I died on Day 11. Histopathology examinations were performed on this animal at the time of the Month 6 interim report.

Summary

Plasma concentrations of E2020 were determined in the chronic toxicity study (Project No. 89-3498) on repeated oral administration for 12 months in beagle dogs. The dose levels were 0.6, 2 and 5 mg/kg/day of E2020. Blood samples were collected from 6 dogs (3 males and 3 females) in each dosage group at pre-dosing and 1, 2, 4, 8 and 24 hours after administration on Days 1, 28, 182 and 364 of dosing. The mean values of Cmax, Cmin, $AUC_{0\text{-}24h}$ (area under the curve), and tmax for males and females combined are summarized below:

Group	Day	Cmax (ng/ml)	Cmin (ng/ml)	$AUC_{0\text{-}24h}$ (ng.hr/ml)	tmax (hour)
0.6 mg/kg	1	3.76 ±2.25	N.D. ±0.00	11.79 ±4.10	3.2 ±2.8
	28	5.09 ±1.84	N.D. ±0.00	20.62 ±11.19	1.7 ±1.2
	182	7.18 ±3.04	N.D. ±0.00	23.65 ±12.85	1.5 ±0.5
	364	8.02 ±4.69	N.D. ±0.00	39.54 ±17.02	2.0 ±1.1
2.0 mg/kg	1	19.48 ±14.70	N.D. ±0.00	69.71 ±39.88	1.8 ±1.2
	28	40.88 ±22.71	N.D. ±0.76	248.65 ±102.38	1.8 ±1.2
	182	52.37 ±21.97	1.40 ±1.20	310.26 ±88.12	1.8 ±1.2
	364	59.80 ±23.27	3.14 ±1.13	404.25 ±82.06	3.2 ±2.8
5.0 mg/kg	1	90.44 ±59.98	1.94 ±1.80	538.34 ±289.98	2.8 ±2.8
	28	246.92 ±65.03	17.68 ±9.22	1926.72 ±532.35	1.8 ±1.2
	182	296.73 ±63.28	38.85 ±30.39	2740.38 ±1109.53	1.5 ±0.6
	364	303.06 ±51.45	56.96 ±35.80	3336.35 ±1194.34	2.2 ±1.0

N.D.:Not Detected (Limit of quantification was 1 ng/ml). Mean±S.D.

Cmax and AUC clearly increased with increasing dosage, and these increases appeared to be more than dose-proportional.

Mean values of Cmax and AUC s increased approximately 2 to 4 times between Day 1 and Day 28 of dosing; however, these changes remained within 2-fold between Day 28 and Day 364 of dosing. The mean plasma concentrations of E2020 reached tmax 1.5 to 3.2 hours after dosing. No sex differences in toxicokinetic parameters were found in all dosage groups.

16 b

*** PATH/TOX SYSTEM OUTPUT ***
A CHRONIC (12 MONTH) ORAL TOXICITY STUDY
OF E2020 IN THE DOG VIA CAPSULE ADMINISTRATION
MICROSCOPIC FINDINGS --- EXPANDED INCIDENCE SUMMARY

TABLE INCLUDES:
SEX=ALL;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=ALL;FIND=ALL;SUBSET=ALL

ORGAN/TISSUE EXAMINED	SEX	MALE				FEMALE			
	GROUP	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
	NUMBER	6	6	6	6	6	6	6	6
** TOP OF LIST **									
LUNGS	NUMBER EXAMINED	6	6	6	6	6	6	6	6
--ALVEOLI:EMPHYSEMA	1>	0	2	0	0	2	0	2	0
	2>	3	1	2	2	0	0	2	4
	3>	0	0	0	0	0	0	1	0
	TL>	3	3	2	2	2	0	5	4
	MN>	2.00	1.33	2.00	2.00	1.00	2.00	1.80	2.00
MEDIASTINAL LN	NUMBER EXAMINED	6	6	6	6	5	6	5	6
--ACUTE/SUBACUTE INFLAMMATION	1>	2	0	1	3	0	1	0	0
	2>	1	1	1	0	0	0	1	3
	TL>	3	1	2	3	0	1	1	3
	MN>	1.33	2.00	1.50	1.00	0.00	1.00	2.00	2.00
LIVER	NUMBER EXAMINED	6	6	6	6	6	6	6	6
--PERIVASCULAR/PERIPORTAL LYMPHOCYTES	1>	4	4	3	2	2	5	4	0
	2>	0	0	0	0	2	1	0	0
	3>	0	0	0	0	0	0	0	0
	TL>	4	4	3	2	3	6	4	0
	MN>	1.00	1.00	1.00	1.00	1.67	1.17	1.00	1.00
THYMUS	NUMBER EXAMINED	6	6	6	6	6	5	6	6
--CYST	1>	0	1	0	0	1	0	2	2
	2>	0	2	4	2	0	2	1	0
	3>	1	0	1	0	0	0	1	0
	TL>	1	3	5	2	1	2	4	2
	MN>	3.00	1.67	2.20	2.00	1.00	1.50	2.00	1.00
SUBLINGUAL	NUMBER EXAMINED	6	6	6	6	5	6	6	6
--MINERALIZATION	1>	2	2	3	5	4	3	2	4
	TL>	2	2	3	5	4	3	2	4
	MN>	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
** END OF LIST **									

(SEVERITY KEY ON NEXT PAGE)

Pathology Summary

Figure A
Lesion Mean Severity (MN) of Selected Findings

Key

$$MN = \frac{(1)(n_1) + (2)(n_2) + (3)(n_3) + (4)(n_4) + (5)(n_5)}{N_L}$$

n_x - where n = number of animals affected with a lesion of X severity
N_L - number of animals exhibiting a lesion
T_L - total number of lesions
MN - Lesion Mean Severity

Severity Codes
1 - minimal
2 - slight
3 - moderate
4 - marked
5 - severe

16 d

T 21B-1
Table 21B

89-3498

A Chronic (12 Month) Oral Toxicity Study
of E2020 in the Dog via Capsule Administration
Pathology Report

Summary Incidence of Non-Neoplastic Microscopic Findings - Male Dogs
6-Month Sacrifice

KEY TO OBSERVATIONS

U/ = UNILATERAL
B/ = BILATERAL
SC = SPINAL CORD
LN = LYMPH NODE(S)
GLS = GLANDS
SG = SALIVARY GLAND(S)
SUB-MANDIB = SUB-MANDIBULAR

*** PATH/TOX SYSTEM OUTPUT ***
A CHRONIC (12 MONTH) ORAL TOXICITY STUDY
OF E2020 IN THE DOG VIA CAPSULE ADMINISTRATION
MICROSCOPIC FINDINGS --- INCIDENCE SUMMARY

STUDY NUMBER: 893498

T 21B-2
Table 21B (cont.)

TABLE INCLUDES:
SEX=M,GROUP=ALL,SCREEN=ALL,WEEKS=ALL
DEATH=I,FIND=P,SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	2
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		2	1	2	0
CERVICAL SC	NUMBER EXAMINED:	2	2	2	2
THORACIC SC	NUMBER EXAMINED:	2	2	2	2
LUMBAR SC	NUMBER EXAMINED:	2	2	2	2
NERVE	NUMBER EXAMINED:	2	2	2	2
HEART	NUMBER EXAMINED:	2	2	2	2
AORTA	NUMBER EXAMINED:	2	2	2	2
TRACHEA	NUMBER EXAMINED:	2	2	2	2
ESOPHAGUS	NUMBER EXAMINED:	2	2	2	2
LUNGS	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		2	2	2	2
--ALVEOLI:EMPHYSEMA		2	2	1	0
--ALVEOLI:ATELECTASIS		1	1	1	0
--ALVEOLI:MACROPHAGES		1	2	1	1
--ALVEOLI:ERYTHROCYTES		1	1	0	0
--PERIBRONCHIOLAR/PERIVASCULAR LYMPHOCYTES		2	1	1	2
--ALVEOLAR/BRONCHIOLAR EPITHELIAL HYPERPLASIA		1	1	0	2
--FIBROSIS		1	0	0	0
MESENTERIC LN	NUMBER EXAMINED:	2	1	2	2
--SINUS ECTASIA		2	1	2	2
--ERYTHROCYTES IN SINUS		2	1	1	2
--EDEMA		2	1	2	2
--LYMPHOCYTOLYSIS		2	1	0	2
--CONGESTION		1	0	0	0
--RETICULOENDOTHELIAL HYPERPLASIA		0	0	2	0
MEDIASTINAL LN	NUMBER EXAMINED:	2	2	2	2
--SINUS ECTASIA		2	2	2	2

** CONTINUED ON NEXT PAGE **

T 21B-3
Table 21B (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
	SEX:	-------MALE-------			
	GROUP:	-1-	-2-	-3-	-4-
	NUMBER:	2	2	2	2
** FROM PREVIOUS PAGE **					
MEDIASTINAL LN	NUMBER EXAMINED:	2	2	2	2
--RETICULOENDOTHELIAL CELLS:PIGMENT		1	1	0	0
--LYMPHOCYTOLYSIS		1	1	2	0
--ERYTHROCYTES IN SINUS		1	0	1	1
--ACUTE/SUBACUTE INFLAMMATION		0	0	2	1
--RETICULOENDOTHELIAL HYPERPLASIA		0	1	1	0
--EDEMA		0	1	0	0
LIVER	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		2	2	2	2
--PERIVASCULAR/PERIPORTAL LYMPHOCYTES		2	2	1	1
--CLOUDY SWELLING		2	2	2	2
--GRANULOMATOUS INFLAMMATION		2	1	2	1
--SUBACUTE/CHRONIC INFLAMMATION		1	0	0	0
--FENSION LIPIDOSIS		1	0	0	0
--RETICULOENDOTHELIAL CELLS:PIGMENT		0	0	1	0
GALLBLADDER	NUMBER EXAMINED:	2	2	2	2
THYMUS	NUMBER EXAMINED:	2	2	2	2
--FREE BLOOD AROUND THYMUS		1	0	0	0
--CONGESTION		1	1	1	1
--LYMPHOCYTOLYSIS		2	2	2	1
--CYST		0	1	1	1
--INVOLUTION		0	1	0	0
SPLEEN	NUMBER EXAMINED:	2	2	2	2
--EXTRAMEDULLARY HEMATOPOIESIS		2	2	2	2
--RETICULOENDOTHELIAL CELLS:PIGMENT		2	2	2	2
SUB-MANDIB SG	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		2	1	1	2
--LYMPHOCYTES		1	0	2	1
PAROTID SG	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		2	0	1	1
--LYMPHOCYTES		2	0	1	1

T 21B-4
Table 21B (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=P;SUBSET=T

ORGAN	D FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
		SEX:	-------MALE-------			
		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	2	2	2	2
SUBLINGUAL		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		1	1	1	0
	--LYMPHOCYTES		2	0	2	1
	--MINERALIZATION		0	0	1	2
STOMACH		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		2	0	1	1
DUODENUM		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		0	1	1	0
PANCREAS		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		2	0	2	0
	--ACUTE/SUBACUTE INFLAMMATION		1	0	0	0
	--LYMPHOCYTES		0	1	0	0
JEJUNUM		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		2	0	1	1
ILEUM		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		0	0	0	1
CECUM		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		1	0	0	0
COLON		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		0	2	0	0
RECTUM		NUMBER EXAMINED:	2	2	2	2
KIDNEYS		NUMBER EXAMINED:	2	2	2	2
	--B/ CONGESTION		2	2	2	2
	--B/ MINERALIZATION		2	2	2	2
	--U/ ACUTE/SUBACUTE INFLAMMATION		1	0	0	0
	--U/ SUBACUTE/CHRONIC INFLAMMATION		0	0	1	0
URINARY BLADDER		NUMBER EXAMINED:	2	2	2	2
	--CONGESTION		0	1	0	0

T 218-5
Table 218 (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	2
TESTES	NUMBER EXAMINED:	2	2	2	2
--B/ ATROPHY/DEGENERATION		0	0	0	1
EPIDIDYMIDES	NUMBER EXAMINED:	2	2	2	2
--U/ SPERMATIC GRANULOMA		1	0	0	0
PROSTATE	NUMBER EXAMINED:	2	2	2	2
ADRENAL GLANDS	NUMBER EXAMINED:	2	2	2	2
--U/ ZONA GLOMERULOSA:VACUOLES		0	0	1	0
--B/ ZONA GLOMERULOSA:VACUOLES		2	1	1	0
--U/ SUBCAPSULAR CORTICAL NODULE		0	0	0	1
--B/ CONGESTION		0	1	0	0
PITUITARY GLAND	NUMBER EXAMINED:	2	2	2	2
--PARS DISTALIS:CYST		1	0	1	0
THYROID GLAND	NUMBER EXAMINED:	1	2	2	2
PARATHYROID GLS	NUMBER EXAMINED:	1	2	2	2
--U/ CYST		0	1	0	0
EYES	NUMBER EXAMINED:	2	2	2	2
--B/ CONGESTION		0	1	0	0
OPTIC NERVE	NUMBER EXAMINED:	2	2	2	2
--MYELIN DEGENERATION/VACUOLES		0	0	1	0
SKIN	NUMBER EXAMINED:	2	2	2	2
MAMMARY GLAND	NUMBER EXAMINED:	2	2	1	2
MUSCLE	NUMBER EXAMINED:	2	2	2	2
TONGUE	NUMBER EXAMINED:	2	2	2	2
BONE STERNUM	NUMBER EXAMINED:	2	2	2	2
MARROW STERNUM	NUMBER EXAMINED:	2	2	2	2

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	2
BONE FEMUR	NUMBER EXAMINED:	2	2	2	2
MARROW FEMUR	NUMBER EXAMINED:	2	2	2	2
BONE RIB	NUMBER EXAMINED:	2	2	2	2
MARROW RIB	NUMBER EXAMINED:	2	2	2	2

** END OF LIST **

T 21B-6
Table 21B (cont.)

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T 21C-1
Table 21C

89-3498

A Chronic (12 Month) Oral Toxicity Study
of E2020 in the Dog via Capsule Administration
Pathology Report

Summary Incidence of Non-Neoplastic Microscopic Findings - Male Dogs
Terminal Sacrifice

KEY TO OBSERVATIONS

U/ = UNILATERAL
B/ = BILATERAL
SC = SPINAL CORD
LN = LYMPH NODE(S)
GLS = GLANDS
SG = SALIVARY GLAND(S)
MESEN/ = MESENTERY
SUB-MANDIB = SUB-MANDIBULAR
MEDIAST/ = MEDIASTINUM

16 k

T 21C-2
Table 21C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		SEX:	--- NUMBER-OF-ANIMALS-AFFECTED --- MALE			
		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	4	4	4	4
** TOP OF LIST **						
BRAIN	NUMBER EXAMINED:		4	4	4	4
--CONGESTION			3	2	2	1
--ACUTE/SUBACUTE INFLAMMATION			2	0	0	0
CERVICAL SC	NUMBER EXAMINED:		4	4	4	4
THORACIC SC	NUMBER EXAMINED:		4	4	4	4
LUMBAR SC	NUMBER EXAMINED:		4	4	4	4
NERVE	NUMBER EXAMINED:		4	4	4	4
HEART	NUMBER EXAMINED:		4	4	4	4
AORTA	NUMBER EXAMINED:		4	4	4	4
TRACHEA	NUMBER EXAMINED:		4	4	4	4
--CONGESTION			2	0	0	0
ESOPHAGUS	NUMBER EXAMINED:		4	4	4	4
LUNGS	NUMBER EXAMINED:		4	4	4	4
--CONGESTION			4	4	4	4
--ALVEOLI:EMPHYSEMA			1	1	1	2
--ALVEOLI:MACROPHAGES			4	2	3	3
--ALVEOLI:ERYTHROCYTES			3	3	3	2
--PERIBRONCHIOLAR/PERIVASCULAR LYMPHOCYTES			1	1	3	1
--ALVEOLAR/BRONCHIOLAR EPITHELIAL HYPERPLASIA			4	2	2	3
--FIBROSIS			0	1	1	0
--RETICULOENDOTHELIAL CELLS:PIGMENT			0	1	0	0
--SEROSA:SUBACUTE/CHRONIC INFLAMMATION			0	0	1	0
MESENTERIC LN	NUMBER EXAMINED:		4	4	4	4
--SINUS ECTASIA			4	4	3	4
--ERYTHROCYTES IN SINUS			2	2	1	4
--EDEMA			0	2	0	0
--LYMPHOCYTOLYSIS			4	3	3	0
--RETICULOENDOTHELIAL HYPERPLASIA			4	2	4	3
--LYMPHOID HYPERPLASIA			1	0	0	0

T 21C-3
Table 21C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		4	4	4	4
MEDIASTINAL LN	NUMBER EXAMINED:	4	4	4	4
--SINUS ECTASIA		4	4	4	4
--RETICULOENDOTHELIAL CELLS:PIGMENT		3	1	0	1
--LYMPHOCYTOLYSIS		1	2	1	0
--ERYTHROCYTES IN SINUS		4	2	1	2
--ACUTE/SUBACUTE INFLAMMATION		3	1	0	2
--RETICULOENDOTHELIAL HYPERPLASIA		1	0	1	1
--PLASMA CELL HYPERPLASIA		0	1	0	1
--LYMPHOID HYPERPLASIA		1	0	0	0
LIVER	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		4	4	4	4
--PERIVASCULAR/PERIPORTAL LYMPHOCYTES		2	2	2	1
--CLOUDY SWELLING		4	3	4	4
--GRANULOMATOUS INFLAMMATION		4	3	3	4
--ACUTE/SUBACUTE INFLAMMATION		1	0	1	0
--HEPATOCYTES:VACUOLES		1	0	0	0
GALLBLADDER	NUMBER EXAMINED:	4	4	4	4
THYMUS	NUMBER EXAMINED:	4	4	4	4
--FREE BLOOD AROUND THYMUS		1	0	0	2
--CONGESTION		3	2	1	2
--CYST		1	2	4	1
--INVOLUTION		1	0	0	0
--HEMORRHAGE		1	1	0	0
SPLEEN	NUMBER EXAMINED:	4	4	4	4
--EXTRAMEDULLARY HEMATOPOIESIS		3	4	4	4
--RETICULOENDOTHELIAL CELLS:PIGMENT		4	4	3	4
--HEMORRHAGE/CONGESTION		0	0	1	0
--FIBROSIDEROTIC NODULE		1	1	0	1
SUB-MANDIB SG	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		2	0	1	0
--LYMPHOCYTES		4	3	2	0
--SUBACUTE/CHRONIC INFLAMMATION		0	0	0	1

T 21C-4
Table 21C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
	SEX:	-------MALE-------			
	GROUP:	-1-	-2-	-3-	-4-
	NUMBER:	4	4	4	4
PAROTID SG	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		1	1	0	0
--LYMPHOCYTES		4	0	1	1
SUBLINGUAL	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		4	0	1	0
--LYMPHOCYTES		4	1	1	1
--MINERALIZATION		2	2	2	3
STOMACH	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		2	3	1	1
--PARIETAL CELLS VACUOLES		0	0	0	1
DUODENUM	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		0	1	0	0
PANCREAS	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		2	2	0	0
JEJUNUM	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		1	1	0	0
ILEUM	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		0	0	0	1
CECUM	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		1	2	2	2
COLON	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		1	1	1	0
RECTUM	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		1	0	1	1
KIDNEYS	NUMBER EXAMINED:	4	4	4	4
--B/ CONGESTION		4	4	4	3
--B/ MINERALIZATION		4	3	4	4
--U/ ACUTE/SUBACUTE INFLAMMATION		0	1	0	1
--B/ ACUTE/SUBACUTE INFLAMMATION		1	1	0	0

** CONTINUED ON NEXT PAGE **

T 21C-5
Table 21C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
	SEX:	-------MALE-------			
	GROUP:	-1-	-2-	-3-	-4-
	NUMBER:	4	4	4	4
** FROM PREVIOUS PAGE **					
KIDNEYS	NUMBER EXAMINED:	4	4	4	4
--U/ DILATED COLLECTING TUBULES		0	0	1	0
--B/ DILATED COLLECTING TUBULES		1	0	0	0
--B/ CONVOLUTED TUBULE EPITHELIUM:PIGMENT		1	1	2	1
--U/ CONGESTION		0	1	0	0
--U/ CONVOLUTED TUBULE EPITHELIUM:PIGMENT		0	1	0	0
URINARY BLADDER	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		1	0	3	1
TESTES	NUMBER EXAMINED:	4	4	4	4
--B/ ATROPHY/DEGENERATION		0	0	1	0
--U/ ATROPHY/DEGENERATION		2	1	2	1
EPIDIDYMIDES	NUMBER EXAMINED:	4	4	4	4
--U/ SPERMATIC GRANULOMA		0	1	0	0
--B/ INTERSTITIAL LYMPHOCYTES		1	0	2	0
--U/ INTERSTITIAL LYMPHOCYTES		0	2	0	0
PROSTATE	NUMBER EXAMINED:	4	4	4	4
--CONGESTION		2	0	0	0
--ACUTE/SUBACUTE INFLAMMATION		0	1	0	0
--LYMPHOCYTES		0	0	0	1
ADRENAL GLANDS	NUMBER EXAMINED:	4	4	4	4
--U/ ZONA GLOMERULOSA:VACUOLES		0	0	1	0
--B/ ZONA GLOMERULOSA:VACUOLES		0	1	0	0
--B/ SUBCAPSULAR CORTICAL NODULE		0	1	0	0
--B/ CONGESTION		2	1	0	0
--U/ CONGESTION		1	0	0	0
PITUITARY GLAND	NUMBER EXAMINED:	4	4	4	4
--PARS DISTALIS:CYST		0	0	1	1
--PARS INTERMEDIUS:CYST		0	1	0	1
--PARS NERVOSA:CYST		0	1	0	0
THYROID GLAND	NUMBER EXAMINED:	4	4	4	4
--CYST		0	0	1	0

T 21C-6
Table 21C (cont.)

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		-------MALE-------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		4	4	4	4
PARATHYROID GLS.	NUMBER EXAMINED:	4	3	4	4
--U/ CYST		0	1	1	1
--B/ CYST		0	0	1	0
EYES	NUMBER EXAMINED:	4	4	4	4
OPTIC NERVE	NUMBER EXAMINED:	4	4	4	4
SKIN	NUMBER EXAMINED:	4	4	4	4
--ACUTE/SUBACUTE INFLAMMATION		0	1	0	0
--GRANULOMATOUS INFLAMMATION		0	0	1	0
MAMMARY GLAND	NUMBER EXAMINED:	2	2	3	1
MUSCLE	NUMBER EXAMINED:	4	4	4	4
TONGUE	NUMBER EXAMINED:	4	4	4	4
BONE STERNUM	NUMBER EXAMINED:	4	4	4	4
MARROW STERNUM	NUMBER EXAMINED:	4	4	4	4
BONE FEMUR	NUMBER EXAMINED:	4	4	4	4
MARROW FEMUR	NUMBER EXAMINED:	4	4	4	4
BONE RIB	NUMBER EXAMINED:	4	4	4	4
MARROW RIB	NUMBER EXAMINED:	4	4	4	4
MESEN/PERITONEUM	NUMBER EXAMINED:	1	0	0	0
MEDIAST/PLEURA	NUMBER EXAMINED:	1	0	0	0
--SUBACUTE/CHRONIC INFLAMMATION		1	0	0	0
EAR	NUMBER EXAMINED:	0	1	1	0
--CONGESTION		0	1	1	0
--NECROSIS		0	1	0	0
--SURFACE DEBRIS/INFLAMMATORY CELLS		0	1	1	0

** CONTINUED ON NEXT PAGE **

TABLE INCLUDES:
SEX=M;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
	SEX:	-------MALE-------			
	GROUP:	-1-	-2-	-3-	-4-
	NUMBER:	4	4	4	4
** FROM PREVIOUS PAGE **					
EAR ...	... NUMBER EXAMINED:	0	1	1	0
--ACUTE/SUBACUTE INFLAMMATION		0	1	1	0

** END OF LIST **

T 21C-7
Table 21C (cont.)

89-3498

A Chronic (12 Month) Oral Toxicity Study
of E2020 in the Dog via Capsule Administration
Pathology Report

Summary Incidence of Non-Neoplastic Microscopic Findings - Female Dogs
Unscheduled Deaths

KEY TO OBSERVATIONS

U/ = UNILATERAL
B/ = BILATERAL
SC = SPINAL CORD
LN = LYMPH NODE(S)
GLS = GLANDS
SG = SALIVARY GLAND(S)
SUB-MANDIB = SUB-MANDIBULAR

T 22A-2
Table 22A (cont.)

TABLE INCLUDES:
SEX=F;GROUP=1;SCREEN=ALL;WEEKS=1-27
DEATH=D;FIXD=P;SUBSET=T

SEX: FEMALE
GROUP: -1-

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---
	NUMBER:	1
** TOP OF LIST **		
BRAIN	NUMBER EXAMINED:	1
--CONGESTION		1
CERVICAL SC	NUMBER EXAMINED:	1
THORACIC SC	NUMBER EXAMINED:	1
--CONGESTION		1
--HEMORRHAGE		1
LUMBAR SC	NUMBER EXAMINED:	1
NERVE	NUMBER EXAMINED:	1
HEART	NUMBER EXAMINED:	1
AORTA	NUMBER EXAMINED:	1
TRACHEA	NUMBER EXAMINED:	1
--MUCUS/DEBRIS IN LUMEN		1
--CONGESTION		1
ESOPHAGUS	NUMBER EXAMINED:	1
LUNGS	NUMBER EXAMINED:	1
--CONGESTION		1
--ALVEOLI:ERYTHROCYTES		1
--PERIBRONCHIOLAR/PERIVASCULAR LYMPHOCYTES		1
MESENTERIC LN	NUMBER EXAMINED:	1
--SINUS ECTASIA		1
--LYMPHOCYTOLYSIS		1
--RETICULOENDOTHELIAL HYPERPLASIA		1
LIVER	NUMBER EXAMINED:	1
--CONGESTION		1
--PERIVASCULAR/PERIPORTAL LYMPHOCYTES		1
--CLOUDY SWELLING		1
--RETICULOENDOTHELIAL CELLS:PIGMENT		1

T 22A-3
Table 22A (cont.)

TABLE INCLUDES:
SEX=F;GROUP=1;SCREEN=ALL;WEEKS=1-27
DEATH=D;FIND=P;SUBSET=T

SEX: FEMALE
GROUP: -1-

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---
	NUMBER:	1
GALLBLADDER	NUMBER EXAMINED:	1
THYMUS	NUMBER EXAMINED:	1
--CONGESTION		1
--LYMPHOCYTOLYSIS		1
SPLEEN	NUMBER EXAMINED:	1
--EXTRAMEDULLARY HEMATOPOIESIS		1
--RETICULOENDOTHELIAL CELLS:PIGMENT		1
--HEMORRHAGE/CONGESTION		1
SUB-MANDIB SG	NUMBER EXAMINED:	1
--CONGESTION		1
--LYMPHOCYTES		1
STOMACH	NUMBER EXAMINED:	1
--CONGESTION		1
DUODENUM	NUMBER EXAMINED:	1
--CONGESTION		1
PANCREAS	NUMBER EXAMINED:	1
--CONGESTION		1
JEJUNUM	NUMBER EXAMINED:	1
--CONGESTION		1
ILEUM	NUMBER EXAMINED:	1
--CONGESTION		1
CECUM	NUMBER EXAMINED:	1
--CONGESTION		1
COLON	NUMBER EXAMINED:	1
--CONGESTION		1
RECTUM	NUMBER EXAMINED:	1
--CONGESTION		1

T 22A-4
Table 22A (cont.)

TABLE INCLUDES:
SEX=F;GROUP=1;SCREEN=ALL;WEEKS=1-27
DEATH=D;FIND=P;SUBSET=T

SEX: FEMALE
GROUP: -1-

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED --- NUMBER: 1
KIDNEYS	NUMBER EXAMINED:	1
--B/ CONGESTION		1
--B/ MINERALIZATION		1
URINARY BLADDER	NUMBER EXAMINED:	1
--CONGESTION		1
OVARIES	NUMBER EXAMINED:	1
UTERUS	NUMBER EXAMINED:	1
VAGINA	NUMBER EXAMINED:	1
ADRENAL GLANDS	NUMBER EXAMINED:	1
--B/ ZONA GLOMERULOSA VACUOLES		1
--B/ CONGESTION		1
PITUITARY GLAND	NUMBER EXAMINED:	1
THYROID GLAND	NUMBER EXAMINED:	1
PARATHYROID GLS	NUMBER EXAMINED:	1
EYES	NUMBER EXAMINED:	1
--U/ CONGESTION		1
OPTIC NERVE	NUMBER EXAMINED:	1
SKIN	NUMBER EXAMINED:	1
MAMMARY GLAND	NUMBER EXAMINED:	1
MUSCLE	NUMBER EXAMINED:	1
TONGUE	NUMBER EXAMINED:	1
BONE STERNUM	NUMBER EXAMINED:	1
MARROW STERNUM	NUMBER EXAMINED:	1

TABLE INCLUDES:
SEX=F;GROUP=1;SCREEN=ALL;WEEKS=1-27
DEATH=D;FIND=P;SUBSET=T

SEX: FEMALE
GROUP: -1-

ORGAN AND FINDING DESCRIPTION		NUMBER: 1
		--- NUMBER OF ANIMALS AFFECTED ---
BONE FEMUR	NUMBER EXAMINED:	1
MARROW FEMUR	NUMBER EXAMINED:	1
BONE RIB	NUMBER EXAMINED:	1
MARROW RIB	NUMBER EXAMINED:	1

** END OF LIST **

T 22A-5
Table 22A (cont.)

T 22B-1
Table 22B

89-3498

A Chronic (12 Month) Oral Toxicity Study
of E2020 in the Dog via Capsule Administration
Pathology Report

Summary Incidence of Non-Neoplastic Microscopic Findings - Female Dogs
6-Month Sacrifice

KEY TO OBSERVATIONS

U/ = UNILATERAL
B/ = BILATERAL
SC = SPINAL CORD
LN = LYMPH NODE(S)
GLS = GLANDS

T 22B-2
Table 22B (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	2
** TOP OF LIST **					
BRAIN	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		2	1	1	0
--ACUTE/SUBACUTE INFLAMMATION		0	1	0	0
CERVICAL SC	NUMBER EXAMINED:	2	2	2	2
THORACIC SC	NUMBER EXAMINED:	2	2	2	2
LUMBAR SC	NUMBER EXAMINED:	2	2	2	2
NERVE	NUMBER EXAMINED:	2	2	2	1
HEART	NUMBER EXAMINED:	2	2	2	2
--MINERALIZATION		0	0	1	0
AORTA	NUMBER EXAMINED:	2	2	2	2
TRACHEA	NUMBER EXAMINED:	2	2	2	2
ESOPHAGUS	NUMBER EXAMINED:	2	2	2	2
LUNGS	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		2	2	2	2
--ALVEOLI:EMPHYSEMA		1	2	2	2
--ALVEOLI:MACROPHAGES		2	2	0	1
--ALVEOLI:ERYTHROCYTES		2	0	0	0
--PERIBRONCHIOLAR/PERIVASCULAR LYMPHOCYTES		1	1	2	1
--ALVEOLAR/BRONCHIOLAR EPITHELIAL HYPERPLASIA		0	2	0	1
--FIBROSIS		0	1	0	1
--ALVEOLI:NEUTROPHILS		0	0	0	1
MESENTERIC LN	NUMBER EXAMINED:	2	2	2	2
--SINUS ECTASIA		2	2	2	2
--ERYTHROCYTES IN SINUS		1	0	0	0
--EDEMA		1	0	0	1
--LYMPHOCYTOLYSIS		2	0	0	1
--RETICULOENDOTHELIAL HYPERPLASIA		0	0	1	0

T 22B-3
Table 22B (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	2
MEDIASTINAL LN	NUMBER EXAMINED:	2	2	2	2
--SINUS ECTASIA		2	2	2	2
--RETICULOENDOTHELIAL CELLS:PIGMENT		1	1	0	1
--LYMPHOCYTOLYSIS		2	2	0	0
--ACUTE/SUBACUTE INFLAMMATION		0	1	0	1
--RETICULOENDOTHELIAL HYPERPLASIA		1	0	0	0
--EDEMA		1	0	0	0
LIVER	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		2	2	2	2
--PERIVASCULAR/PERIPORTAL LYMPHOCYTES		2	2	2	1
--CLOUDY SWELLING		2	2	2	2
--GRANULOMATOUS INFLAMMATION		2	1	2	1
--ACUTE/SUBACUTE INFLAMMATION		1	0	0	0
GALLBLADDER	NUMBER EXAMINED:	2	2	2	2
--CYSTIC MUCINOUS HYPERPLASIA		0	0	1	0
THYMUS	NUMBER EXAMINED:	2	2	2	2
--FREE BLOOD AROUND THYMUS		0	0	1	1
--CONGESTION		2	2	0	0
--LYMPHOCYTOLYSIS		2	0	0	0
--CYST		0	1	2	1
SPLEEN	NUMBER EXAMINED:	2	2	2	2
--EXTRAMEDULLARY HEMATOPOIESIS		2	2	1	2
--RETICULOENDOTHELIAL CELLS:PIGMENT		2	2	1	.
SUB-MANDIB SG	NUMBER EXAMINED:	2	2	2	2
--LYMPHOCYTES		2	0	1	1
--MINERALIZATION		0	1	0	0
PAROTID SG	NUMBER EXAMINED:	2	2	2	2
--LYMPHOCYTES		1	0	0	1
SUBLINGUAL	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		1	0	0	1
--LYMPHOCYTES		1	0	1	2
--MINERALIZATION		1	1	1	0

T 22B-4
Table 22B (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=I;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	2
STOMACH	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		0	1	0	0
DUODENUM	NUMBER EXAMINED:	2	2	2	2
PANCREAS	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		1	0	0	0
JEJUNUM	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		0	0	1	0
ILEUM	NUMBER EXAMINED:	2	2	2	2
CECUM	NUMBER EXAMINED:	2	2	2	2
COLON	NUMBER EXAMINED:	2	2	2	2
--CONGESTION		0	0	1	0
RECTUM	NUMBER EXAMINED:	2	2	2	2
KIDNEYS	NUMBER EXAMINED:	2	2	2	2
--B/ CONGESTION		2	2	2	2
--U/ MINERALIZATION		0	0	0	1
--B/ MINERALIZATION		2	2	2	1
URINARY BLADDER	NUMBER EXAMINED:	2	2	2	2
OVARIES	NUMBER EXAMINED:	2	2	2	2
--U/ MINERALIZATION		0	0	0	1
UTERUS	NUMBER EXAMINED:	2	2	2	2
--PSEUDOCYESIS		0	0	0	1
--ENDOMETRIAL GLANDS:HYPERPLASIA		0	1	0	0
VAGINA	NUMBER EXAMINED:	2	2	2	2
ADRENAL GLANDS	NUMBER EXAMINED:	2	2	2	2
--U/ ZONA GLOMERULOSA:VACUOLES		0	0	1	0
--B/ ZONA GLOMERULOSA:VACUOLES		0	1	0	0

** CONTINUED ON NEXT PAGE **

T 22B-5
Table 22B (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1,FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		2	2	2	2
** FROM PREVIOUS PAGE **					
ADRENAL GLANDS	NUMBER EXAMINED:	2	2	2	2
--U/ SUBCAPSULAR CORTICAL NODULE		1	0	1	0
--B/ SUBCAPSULAR CORTICAL NODULE		0	0	1	0
PITUITARY GLAND	NUMBER EXAMINED:	2	2	2	2
--PARS DISTALIS:CYST		0	1	1	0
THYROID GLAND	NUMBER EXAMINED:	2	2	1	2
--MINERALIZATION		0	0	0	1
PARATHYROID GLS	NUMBER EXAMINED:	2	2	1	2
--U/ CYST		0	0	1	2
EYES	NUMBER EXAMINED:	2	2	2	2
OPTIC NERVE	NUMBER EXAMINED:	2	2	2	2
SKIN	NUMBER EXAMINED:	2	2	2	2
MAMMARY GLAND	NUMBER EXAMINED:	2	2	2	2
--DUCT/ACINI ECTASIA		2	1	2	2
--DUCTAL/ACINAR HYPERPLASIA		1	1	0	0
MUSCLE	NUMBER EXAMINED:	2	2	2	1
TONGUE	NUMBER EXAMINED:	2	2	2	2
BONE STERNUM	NUMBER EXAMINED:	2	2	2	2
MARROW STERNUM	NUMBER EXAMINED:	2	2	2	2
BONE FEMUR	NUMBER EXAMINED:	2	2	2	1
MARROW FEMUR	NUMBER EXAMINED:	2	2	2	1
BONE RIB	NUMBER EXAMINED:	2	2	2	2
MARROW RIB	NUMBER EXAMINED:	2	2	2	2

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=1;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
	SEX:	------FEMALE------			
	GROUP:	-1-	-2-	-3-	-4-
	NUMBER:	2	2	2	2
EXTREMITIES	NUMBER EXAMINED:	0	1	0	0
--CONGESTION		0	1	0	0
--ACUTE/SUBACUTE INFLAMMATION		0	1	0	0

** END OF LIST **

T 22B-6
Table 22B (cont.)

T 22C-1
Table 22C

89-3498

A Chronic (12 Month) Oral Toxicity Study
of E2020 in the Dog via Capsule Administration
Pathology Report

Summary Incidence of Non-Neoplastic Microscopic Findings - Female Dogs
Terminal Sacrifice

KEY TO OBSERVATIONS

U/ = UNILATERAL
B/ = BILATERAL
SC = SPINAL CORD
LN = LYMPH NODE(S)
GLS = GLANDS
CAV = CAVITY
SG = SALIVARY GLAND(S)
SUB-MANDIB = SUB-MANDIBULAR

16 cc

T 22C-2
Table 22C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		SEX:	FEMALE			
		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	3	4	4	4
** TOP OF LIST **						
BRAIN	NUMBER EXAMINED:		3	4	4	4
--CONGESTION			1	2	4	3
--MENINGES:GRANULOMATOUS INFLAMMATION			0	0	0	1
CERVICAL SC	NUMBER EXAMINED:		3	4	4	4
THORACIC SC	NUMBER EXAMINED:		3	4	4	4
LUMBAR SC	NUMBER EXAMINED:		3	4	4	4
NERVE	NUMBER EXAMINED:		3	4	4	4
HEART	NUMBER EXAMINED:		3	4	4	4
--ARTERY:PERIARTERITIS NODOSA			0	0	1	0
AORTA	NUMBER EXAMINED:		3	4	4	4
TRACHEA	NUMBER EXAMINED:		3	4	4	4
--CONGESTION			0	1	1	0
ESOPHAGUS	NUMBER EXAMINED:		3	4	4	4
LUNGS	NUMBER EXAMINED:		3	4	4	4
--CONGESTION			3	4	4	4
--ALVEOLI:EMPHYSEMA			1	2	2	2
--ALVEOLI:ATELECTASIS			0	1	1	1
--ALVEOLI:MACROPHAGES			2	3	4	3
--ALVEOLI:ERYTHROCYTES			2	0	4	2
--PERIBRONCHIOLAR/PERIVASCULAR LYMPHOCYTES			1	2	2	2
--ALVEOLAR/BRONCHIOLAR EPITHELIAL HYPERPLASIA			3	2	2	2
--FIBROSIS			0	1	1	2
--SEROSA:SUBACUTE/CHRONIC INFLAMMATION			0	0	1	1
MESENTERIC LN	NUMBER EXAMINED:		3	4	4	4
--SINUS ECTASIA			3	4	4	4
--ERYTHROCYTES IN SINUS			1	2	4	1
--EDEMA			3	2	0	0
--LYMPHOCYTOLYSIS			2	0	1	0
--RETICULOENDOTHELIAL HYPERPLASIA			3	3	2	1

T 22C-3
Table 22C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T,FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
	SEX:	FEMALE			
	GROUP:	-1-	-2-	-3-	-4-
	NUMBER:	3	4	4	4
MEDIASTINAL LN	NUMBER EXAMINED:	3	4	4	4
--SINUS ECTASIA		2	4	4	4
--RETICULOENDOTHELIAL CELLS:PIGMENT		2	2	3	2
--LYMPHOCYTOLYSIS		1	0	0	1
--ERYTHROCYTES IN SINUS		3	2	2	2
--ACUTE/SUBACUTE INFLAMMATION		0	0	1	2
--RETICULOENDOTHELIAL HYPERPLASIA		1	1	0	1
--PLASMA CELL HYPERPLASIA		0	2	0	0
--LYMPHOID HYPERPLASIA		1	0	0	0
LIVER	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		3	4	4	4
--PERIVASCULAR/PERIPORTAL LYMPHOCYTES		0	4	2	4
--CLOUDY SWELLING		3	3	4	3
--GRANULOMATOUS INFLAMMATION		3	3	3	4
--SUBACUTE/CHRONIC INFLAMMATION		0	0	0	1
--ACUTE/SUBACUTE INFLAMMATION		0	0	1	0
--RETICULOENDOTHELIAL CELLS:PIGMENT		0	0	1	0
--HEPATOCYTES:VACUOLES		1	0	1	1
--EXTRAMEDULLARY HEMATOPOIESIS		1	0	0	0
GALLBLADDER	NUMBER EXAMINED:	3	4	4	4
THYMUS	NUMBER EXAMINED:	3	3	4	4
--FREE BLOOD AROUND THYMUS		1	0	1	0
--CONGESTION		2	3	0	3
--CYST		1	3	4	4
--INVOLUTION		1	0	1	1
--HEMORRHAGE		0	1	0	0
--ECTOPIC THYROID TISSUE		0	0	1	0
SPLEEN	NUMBER EXAMINED:	3	4	4	4
--EXTRAMEDULLARY HEMATOPOIESIS		3	3	3	3
--RETICULOENDOTHELIAL CELLS:PIGMENT		3	4	4	4
--HEMORRHAGE/CONGESTION		0	0	0	1
--FIBROSIDEROTIC NODULE		0	1	1	1
--SEROSA:SUBACUTE/CHRONIC INFLAMMATION		0	0	1	0

T 22C-4
Table 22C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER-OF-ANIMALS-AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		3	4	4	4
SUB-MANDIB SG	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		0	0	0	2
--LYMPHOCYTES		2	0	2	2
PAROTID SG	NUMBER EXAMINED:	3	4	4	4
--LYMPHOCYTES		2	1	2	2
SUBLINGUAL	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		3	1	0	0
--LYMPHOCYTES		2	2	1	1
--MINERALIZATION		3	2	1	4
STOMACH	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		1	1	1	4
DUODENUM	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		1	0	1	0
--SUBMUCOSA EDEMA		0	0	1	0
PANCREAS	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		0	1	1	2
JEJUNUM	NUMBER EXAMINED:	3	4	4	4
ILEUM	NUMBER EXAMINED:	3	4	4	4
CECUM	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		0	1	2	0
COLON	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		0	1	1	0
RECTUM	NUMBER EXAMINED:	3	4	4	4
KIDNEYS	NUMBER EXAMINED:	3	4	4	4
--B/ CONGESTION		2	3	4	4
--U/ MINERALIZATION		1	2	2	0
--B/ MINERALIZATION		2	2	2	4
--U/ DILATED COLLECTING TUBULES		1	0	0	0

** CONTINUED ON NEXT PAGE **

T 22C-5
Table 22C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T;FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		3	4	4	4
** FROM PREVIOUS PAGE **					
KIDNEYS	NUMBER EXAMINED:	3	4	4	4
--B/ CONVOLUTED TUBULE EPITHELIUM:PIGMENT		0	0	2	0
URINARY BLADDER	NUMBER EXAMINED:	3	4	4	4
--CONGESTION		2	3	2	1
OVARIES	NUMBER EXAMINED:	3	4	4	4
--B/ CYSTIC CORPORA LUTEA		0	1	0	0
UTERUS	NUMBER EXAMINED:	3	4	4	4
--LUMEN DILATED		1	0	0	0
VAGINA	NUMBER EXAMINED:	3	4	4	4
ADRENAL GLANDS	NUMBER EXAMINED:	3	4	4	4
--B/ ZONA GLOMERULOSA:VACUOLES		0	0	1	0
--B/ CONGESTION		0	1	1	2
--U/ CONGESTION		0	2	0	0
PITUITARY GLAND	NUMBER EXAMINED:	3	4	4	4
--PARS DISTALIS:CYST		0	1	2	0
--PARS INTERMEDIUS:CYST		2	0	1	1
--PARS NERVOSA:CYST		0	0	0	1
THYROID GLAND	NUMBER EXAMINED:	3	4	4	4
--MINERALIZATION		0	1	0	0
--LYMPHOCYTES		0	0	1	0
PARATHYROID GLS	NUMBER EXAMINED:	3	4	4	4
--U/ CYST		1	2	1	0
EYES	NUMBER EXAMINED:	3	4	4	4
OPTIC NERVE	NUMBER EXAMINED:	3	4	4	4
SKIN	NUMBER EXAMINED:	3	4	4	4
MAMMARY GLAND	NUMBER EXAMINED:	3	4	4	4
--DUCT/ACINI ECTASIA		3	3	2	2

** CONTINUED ON NEXT PAGE **

T 22C-6
Table 22C (cont.)

TABLE INCLUDES:
SEX=F;GROUP=ALL;SCREEN=ALL;WEEKS=ALL
DEATH=T,FIND=P;SUBSET=T

ORGAN AND FINDING DESCRIPTION		--- NUMBER OF ANIMALS AFFECTED ---			
SEX:		------FEMALE------			
GROUP:		-1-	-2-	-3-	-4-
NUMBER:		3	4	4	4
** FROM PREVIOUS PAGE **					
MAMMARY GLAND	NUMBER EXAMINED:	3	4	4	4
--DUCTAL/ACINAR HYPERPLASIA		1	2	2	2
MUSCLE	NUMBER EXAMINED:	3	4	4	4
TONGUE	NUMBER EXAMINED:	3	4	4	4
--ACUTE/SUBACUTE INFLAMMATION		0	2	0	0
BONE STERNUM	NUMBER EXAMINED:	3	4	4	4
MARROW STERNUM	NUMBER EXAMINED:	3	4	4	4
BONE FEMUR	NUMBER EXAMINED:	3	4	4	4
MARROW FEMUR	NUMBER EXAMINED:	3	4	4	4
BONE RIB	NUMBER EXAMINED:	3	4	4	4
MARROW RIB	NUMBER EXAMINED:	3	4	4	4
GINGIVA	NUMBER EXAMINED:	0	0	0	1
--ACUTE/SUBACUTE INFLAMMATION		0	0	0	1
--NECROSIS		0	0	0	1
** END OF LIST **					

SEGMENT 1 REPRODUCTION IN RATS

A) Method

24 M + 24 F at 0, 1, 3, or 10 mg/kg/day, by gavage.

Dosing in M was from 63 days pre-mating through a 2 week mating period. F were dosed from 14 days pre-mating through either day 7 of gestation (half of the F, which had cesarian section performed on day 20 of gestation) or through day 21 PP (remaining F, which were allowed to deliver naturally).

Four control M were inadvertently dosed with 10 mg/kg of drug on day 19 of dosing.

F_1 offspring were evaluated for weight gain, survival, developmental milestones, and reproductive performance.

Strain: Sprague-Dawley (from Japan SLC Inc., Hamamatsu, Japan)

Drug lot#: 89120401

B) Results

1) Observed signs in Fo

No drug effects

2) Fo mortality

One LD M, 2 HD M, and 1 HD F died, believed to be dosing accidents.

3) Fo bodyweight

Weights of F assigned for natural delivery were generally below control at MD and to a greater extent at HD throughout the gestation and lactation periods; this only achieved statistical significance at HD during the lactation period. (Mean ~ 90-95% of control; see attached table). (Very slight, non-statistically significantly lower bodyweight gains were also seen among HD F sacrificed for C-section during the days of gestation that they were dosed, i.e. days 0-7, and in HD F during the first few days of dosing pre-mating).

There were no clear drug effects in M.

4) Fo food consumption

Statistically significant decreases in HD F during first few days of dosing pre-mating and during the lactation period (Mean 85-90% of control). (Decreases of smaller magnitude were seen in LD F and MD F during lactation period, but

generally not statistically significant). Slight decreases seen in HD F on the first day of gestation, but not statistically significant.

No clear drug effects in M.

5) Fo estrous cycle

Prolonged at HD (see attached table)

6) Fo fertility

No drug effects (see attached table)

7) Other reproductive parameters

(See attached table for parameters measured and results obtained).

No clear or consistent drug effects seen. Fetal wight was statistically significantly increased among HD F sacrificed on day 20 of gestation (see table "13"); however weights of F_1 offspring from HD F allowed to deliver naturally were similar to controls on day 1 PP, and were less than controls (up to 5-10%, but not statistically significant) beginning around 1 week PP. (see tables "15" and "18").

Table 6 Body weight (g) of F0 dams assigned for natural delivery

Dose (mg/kg)		Control	1	3	10
No. of animals examined		9	10	10	10
Days of gestation	0	246.9±4.6	244.1±4.0	238.2±5.6	238.0±5.0
	2	255.7±4.7	252.2±4.2	244.9±5.2	243.1±5.8
	4	262.9±4.6	260.6±4.4	251.2±5.1	249.0±6.4
	6	270.1±4.5	269.9±4.6	260.5±4.1	255.3±6.6
	7	272.9±4.6	273.3±4.5	262.6±4.3	258.6±6.6
	8	277.6±4.8	277.2±4.8	266.7±4.5	261.5±6.5
	10	286.4±4.8	288.5±4.5	277.3±4.7	270.7±7.3
	12	297.6±5.1	299.4±4.5	288.6±4.9	281.0±7.2
	14	305.0±5.4	308.4±5.0	295.9±5.7	287.6±7.2
	16	318.7±5.2	323.2±5.2	312.3±5.5	301.7±7.4
	18	333.8±6.9	342.6±6.6	328.8±9.0	318.9±8.1
	20	362.7±5.7	366.3±6.4	353.8±8.7	342.4±7.6
	0-7	26.0±2.2	29.2±1.9	24.4±2.3	20.6±2.1
	7-20	89.8±3.1	93.0±4.6	91.2±5.7	83.8±2.5
Days of lactation	1	275.2±5.9	276.0±4.9	274.5±5.4	260.8±7.9
	4	286.3±4.4	283.9±3.8	281.2±4.2	263.8±5.9**a
	7	295.8±5.8	290.6±3.8	286.0±4.3	269.8±6.6**a
	10	307.4±5.1	300.9±3.0	297.5±3.7	280.1±6.2**a
	14	310.2±5.2	306.9±3.2	302.7±5.1	282.4±6.3**a
	17	305.8±4.6	305.8±3.5	303.0±4.8	286.6±5.5*a
	21	303.6±5.7	303.4±4.6	297.8±4.3	287.1±5.7
	1-21	28.3±3.7	27.4±5.4	23.3±2.9	26.3±5.1

Data represent Mean ± S.E.
Significantly different from control * $p<0.05$, ** $p<0.01$ a : Dunnett

Table 3 Estrous cycle of F0 female rats

Dose (mg/kg)	Control	1	3	10
No. of animals examined	24	23	24	23
Estrous cycle length (days)	4.0±0.07	4.0±0.03	4.2±0.07	4.4±0.10**a
Total occurence of estrus	88	85	86	77
Estrus (%) with each duration				
1 day	76(86.4)	81(95.3)	74(86.0)	53(68.8)**b
2 days	12(13.6)	4(4.7)	9(10.5)	22(28.6)* b
3 days or more	0(0.0)	0(0.0)	3(3.5)	2(2.6)

Data represent Mean ± S.E.
Significantly different from control * $p<0.05$, ** $p<0.01$ a : Dunnett ranking b : Chi-square

Table 10 Fertility findings of F0 parental animals

Dose (mg/kg)	Control	1	3	10
No. of pairs	24	23	24	21
Cesarean section	12	11	12	11
Natural delivery	12	12	12	10
No. of mated pairs (% of pairs)	23 (95.8)	23 (100.0)	23 (95.8)	21 (100.0)
Cesarean section	12	11	11	11
Natural delivery	11	12	12	10
Days to confirmed mating				
Mean ± S.E.	2.9 ± 0.59	1.9 ± 0.29	2.7 ± 0.55	2.5 ± 0.32
No. of pregnant females (% of mated)	21 (91.3)	21 (91.3)	20 (87.0)	19 (90.5)
Cesarean section	12	11	10	9
Natural delivery	9	10	10	10

Table 11 Delivery findings of F0 dams

Dose (mg/kg)		Control	1	3	10
No. of pregnant females		9	10	10	10
No. of females with live offspring (%)		9 (100.0)	10 (100.0)	10 (100.0)	10 (100.0)
Gestation length (days)	Mean ± S.E	21.3 ± 0.2	21.3 ± 0.2	21.5 ± 0.2	21.7 ± 0.2
No. of implantation scars	Total	132	143	128	137
	Mean ± S.E.	14.7 ± 0.6	14.3 ± 0.6	12.8 ± 1.3	13.7 ± 0.6
No. of delivered offspring	Total	118	130	115	117
	Mean ± S.E.	13.1 ± 0.7	13.0 ± 0.9	11.5 ± 1.2	11.7 ± 0.8
No. of live offspring	Total	118	129	112	116
	Mean ± S.E.	13.1 ± 0 7	12.9 ± 1.0	11.2 ± 1.3	11.6 ± 0.7
Sex ratio		1.11	1.11	0.81	0.81
(male/female)		62/56	68/61	50/62	52/64
No. of dead offspring	Total (%)	0	1(0.8)	3(2.6)	1(0.9)
Live birth ratio [a)]		89.4	90.2	87.5	84.7

a) : (No. of live offspring / No. of implantation scars) × 100

Table 12 Nursing findings of Fo dams

Dose (mg/kg)		Control	1	3	10
No. of females with live offsprig (%)		9	10	10	10
Eating of placenta[a)]	day1	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
Retrieving[a)]	day1	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day2	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day3	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day4	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
Lactation[a)]	day1	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day2	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day3	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day4	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
No cannibalism[a)]	day1	9/9(100.0)	10/10(100.0)	9/10(90.0)	9/10(90.0)
	day2	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day3	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day4	9/9(100.0)	10/10(100.0)	10/10(100.0)	9/10(90.0)
Nursing ratio[b)]	day4	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)
	day21	9/9(100.0)	10/10(100.0)	10/10(100.0)	10/10(100.0)

a): (No. of dams showing each nursing behavior / No. of dams examined) × 100
b): (No. of nursing females / No. of females with live offspring) × 100

Table 13 Cesarean section of F0 dams

Dose (mg/kg)		Control	1	3	10
No. of litters examined		12	11	10	9
No. of corpora lutea	Total	181	164	144	129
	Mean ± S.E.	15.1 ± 0.42	14.9 ± 0.74	14.4 ± 0.45	14.3 ± 0.60
No. of implantations	Total	166	155	139	127
	Mean ± S.E.	13.8 ± 0.55	14.1 ± 0.71	13.9 ± 0.57	14.1 ± 0.59
Implantation ratio		166/181	155/164	139/144	127/129
	Litter Mean(%)	92.1	94.6	96.4	98.5
No. of resorptions and dead fetuses	Total	11	11	7	6
	LitterMean(%)	6.8	7.2	5.1	4.6
No. of live fetuses	Total	155	144	132	121
	Mean ± S.E.	12.9 ± 0.61	13.1 ± 0.74	13.2 ± 0.65	13.4 ± 0.56
Sex ratio		0.82	1.06	1.03	0.92
(male/female)		(70/85)	(74/70)	(67/65)	(58/63)
Fetal body weight (g)	Mean ± S.E.	3.48 ± 0.05	3.57 ± 0.06	3.65 ± 0.08	3.75 ± 0.06**a
No. of fetuses with external anomalies					
	Total	0	0	0	1[a)]
	Litter Mean (%)				0.9

a): proboscis + anophthalmia + astomia + microtia
Significantly different from control ** $p<0.01$ a : Dunnett

Table 14 Viability and external findings of F1 offspring

Dose (mg/kg)		Control	1	3	10
No. of litters examined		9	10	10	10
No. of live offspring					
Day 1	Total [a]	118	129	112	116
	Mean±S.E.	13.1±0.7	12.9±1.0	11.2±1.3	11.6±0.7
Day 4	Total [b]	118	129	112	115
	Mean±S.E.	13.1±0.7	12.9±1.0	11.2±1.3	11.5±0.7
Day 4 [e]	Total [c]	72	78	73	79
	Mean±S.E.	8.0±0.0	7.8±0.2	7.3±0.6	7.9±0.1
Day 21	Total [d]	72	77	72	79
	Mean±S.E.	8.0±0.0	7.7±0.2	7.2±0.6	7.9±0.1
No. of dead offspring					
Day 1		0	1	3	1
Days 2 to 4		0	0	0	1
Days 4 [e] to 21		0	1	1	0
Viability index (%)					
Days 1 to 4 [f]		100.0	100.0	100.0	99.1
Days 4 [e] to 21 [g]		100.0	98.7	98.6	100.0
No. of offspring with external anomalies (%)					
Days 1 to 4		0	0	0	0
Days 4 [e] to 21		0	0	0	0

e) : post-culling f) : (b/a)×100 g) : (d/c)×100

Table 15 Body weight (g) of F1 offspring

Dose (mg/kg)		Control	1	3	10
Males					
No. of litters		9	10	9	10
Days of age	1	6.4±0.2	6.4±0.2	6.2±0.1	6.4±0.2
	4	9.5±0.3	9.2±0.4	9.0±0.2	9.2±0.4
	4a)	9.4±0.3	9.2±0.4	9.0±0.2	9.3±0.4
	7	14.8±0.4	14.2±0.6	14.4±0.3	14.0±0.5
	14	28.4±0.7	27.6±0.8	27.7±0.5	26.3±0.9
	21	43.9±1.4	43.1±1.1	42.6±0.8	40.9±1.4
	1-4	3.1±0.1	2.8±0.2	2.9±0.2	2.8±0.3
	4a)-21	34.4±1.3	34.0±0.8	33.6±0.7	31.7±1.0
Females					
No. of litters		9	10	10	10
Days of age	1	6.0±0.2	6.0±0.2	6.1±0.2	6.1±0.2
	4	9.0±0.3	8.9±0.4	9.1±0.5	9.0±0.4
	4a)	9.1±0.3	8.8±0.4	9.1±0.5	9.1±0.4
	7	14.2±0.4	13.8±0.5	14.2±0.5	13.7±0.4
	14	27.7±0.6	27.0±0.5	26.8±0.5	25.9±0.8
	21	42.5±1.1	41.7±0.9	41.2±0.8	40.3±1.3
	1-4	3.0±0.2	2.9±0.3	3.1±0.3	2.9±0.3
	4a)-21	33.4±1.0	32.9±0.7	32.1±0.5	31.2±1.0

Data represent Mean ± S.E. a) : post-culling

Table 16 Physical and functional development of F1 offspring

Dose (mg/kg)	Control	1	3	10
Males				
No. of litters	9	10	9	10
Day of pinna unfolding	3.4 ± 0.15	3.5 ± 0.18	3.5 ± 0.20	2.8 ± 0.21
Day of incisor eruption	12.2 ± 0.26	12.1 ± 0.32	12.9 ± 0.15	11.8 ± 0.25
Day of eyelid opening	16.5 ± 0.25	16.5 ± 0.27	16.8 ± 0.23	16.5 ± 0.27
Day of palmar grasp	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00
Day of auditory startle	13.5 ± 0.18	13.8 ± 0.24	13.9 ± 0.15	13.5 ± 0.21
Females				
No. of litters	9	10	10	10
Day of pinna unfolding	3.4 ± 0.14	3.4 ± 0.18	3.3 ± 0.24	2.8 ± 0.17
Day of incisor eruption	11.9 ± 0.26	11.8 ± 0.36	12.2 ± 0.40	11.9 ± 0.27
Day of eyelid opening	16.3 ± 0.22	16.5 ± 0.26	16.7 ± 0.29	16.6 ± 0.26
Day of palmar grasp	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00	5.0 ± 0.00
Day of auditory startle	13.4 ± 0.14	13.6 ± 0.21	13.6 ± 0.32	13.4 ± 0.22

Data represent Mean ± S.E.

Table 17 Organ weight of F1 offspring at 21 days of age

Dose (mg/kg)	Control	1	3	10
No. of males	9	10	9	10
Body weight (g)	44.86 ± 1.98	42.56 ± 1.15	42.91 ± 0.89	40.12 ± 1.88
Liver (g)	1.659 ± 0.087 (3.691 ± 0.050)	1.518 ± 0.064 (3.557 ± 0.074)	1.528 ± 0.048 (3.557 ± 0.069)	1.440 ± 0.090 (3.566 ± 0.066)
Kidneys (g)	0.4777 ± 0.0183 (1.0681 ± 0.0169)	0.4426 ± 0.0110 (1.0418 ± 0.0158)	0.4654 ± 0.0140 (1.0851 ± 0.0265)	0.4310 ± 0.0238 (1.0707 ± 0.0202)
Brain (g)	1.4618 ± 0.0162 (3.3033 ± 0.1306)	1.4379 ± 0.0106 (3.3979 ± 0.0829)	1.4373 ± 0.0110 (3.3591 ± 0.0613)	1.4119 ± 0.0216 (3.5749 ± 0.1356)
Testes (g)	0.2149 ± 0.0121 (0.4781 ± 0.0107)	0.2015 ± 0.0080 (0.4729 ± 0.0102)	0.2107 ± 0.0075 (0.4900 ± 0.0099)	0.2016 ± 0.0124 (0.4998 ± 0.0102)
No. of females	9	10	10	10
Body weight (g)	42.36 ± 1.33	42.48 ± 1.26	41.67 ± 1.20	40.50 ± 1.37
Liver (g)	1.556 ± 0.063 (3.667 ± 0.060)	1.590 ± 0.063 (3.734 ± 0.051)	1.551 ± 0.072 (3.709 ± 0.077)	1.486 ± 0.077 (3.654 ± 0.073)
Kidneys (g)	0.4768 ± 0.0156 (1.1263 ± 0.0162)	0.4687 ± 0.0147 (1.1038 ± 0.0156)	0.4842 ± 0.0182 (1.1604 ± 0.0211)	0.4619 ± 0.0201 (1.1400 ± 0.0283)
Brain (g)	1.3997 ± 0.0117 (3.3273 ± 0.0935)	1.3929 ± 0.0171 (3.3000 ± 0.0870)	1.3807 ± 0.0200 (3.3344 ± 0.0938)	1.3828 ± 0.0191 (3.4393 ± 0.0915)
Ovaries (mg)	13.3 ± 0.9 (31.61 ± 2.06)	12.0 ± 0.8 (28.18 ± 1.56)	11.9 ± 1.7 (27. 69 ± 3.38)	12.6 ± 1.2 (30.64 ± 2.11)

Data represent Mean ± S.E. ( ) : (organ weight / body weight) × 100

Table 18 Body weight and body weight gain (g) of F1 offspring

Dose (mg/kg)		Control	1	3	10
No. of males		9	10	9	10
Weeks of age	3	44.2±1.6	43.0±2.1	43.4±1.2	41.0±1.4
	4	107.0±5.4	109.8±6.8	114.3±7.3	98.0±4.8
	5	163.8±6.3	166.6±9.0	171.9±8.5	153.0±6.9
	6	220.4±7.4	220.4±10.9	229.1±9.3	207.1±8.5
	7	278.7±8.8	276.1±12.6	288.8±10.3	262.2±10.3
	8	328.4±8.5	322.6±13.8	338.4±9.8	309.2±11.0
	9	369.2±8.9	359.9±14.8	378.0±9.0	347.0±11.6
	10	397.7±9.3	384.9±15.4	405.7±8.4	370.6±12.6
	11	426.3±10.5	414.9±16.4	431.7±8.3	398.8±13.9
	3-5	119.6±5.9	123.6±7.3	128.4±7.5	112.0±6.4
	5-7	114.9±3.7	109.5±4.1	116.9±2.2	109.2±4.0
	7-11	144.8±5.6	138.8±6.9	140.9±3.7	136.6±5.1
No. of females		9	10	10	10
Weeks of age	3	41.9±1.0	41.7±1.1	41.3±0.9	40.8±1.0
	4	93.8±5.3	98.1±3.9	95.8±4.6	89.5±3.3
	5	131.4±4.3	137.4±5.0	131.5±4.9	128.1±3.4
	6	162.4±5.0	166.7±6.0	160.3±5.6	156.0±4.0
	7	191.9±5.8	191.6±6.8	186.1±5.9	184.8±5.1
	8	217.1±6.4	214.7±6.3	207.7±6.3	206.0±5.5
	9	233.3±7.2	232.4±6.5	222.9±6.7	218.5±5.4
	10	246.8±7.4	247.4±7.1	236.4±7.0	232.0±4.6
	11	256.8±7.1	259.5±8.0	252.3±9.0	247.2±4.8
	3-5	89.6±3.9	95.7±4.5	90.2±4.3	87.3±3.5
	5-7	60.4±3.1	54.2±2.7	54.6±2.6	56.7±2.0
	7-11	64.9±2.1	67.9±2.2	63.3±3.3	62.4±0.9

Data represent Mean ± S.E.

Table 19 Fertility findings of F1 offspring

Dose (mg/kg)		Control	1	3	10
No. of males		9	10	9	10
Day of testicular descent	Mean ± S.E.	22.7 ± 0.17	23.8 ± 0.36	23.7 ± 0.41	23.2 ± 0.25
No. of females		9	10	10	10
Day of vaginal opening	Mean ± S.E.	34.3 ± 0.58	34.6 ± 0.62	34.6 ± 0.87	33.7 ± 0.68
No. of pairs		9	10	9	10
No. of mated pairs (% of pairs)		8 (88.9)	10 (100.0)	9 (100.0)	10 (100.0)
Days to confirmed mating	Mean ± S.E.	1.9 ± 0.40	4.3 ± 0.99	3.1 ± 1.25	2.5 ± 0.90
No. of pregnant females (%)		8 (100.0)	9 (90.0)	9 (100.0)	9 (90.0)

Table 20 Body weight (g) of F1 dams

Dose (mg/kg)		Control	1	3	10
No. of pregnant females		8	9	9	9
Days of gestation	0	263.6 ± 6.9	274.6 ± 8.4	256.1 ± 9.0	257.3 ± 4.9
	7	294.4 ± 5.6	307.3 ± 7.9	279.9 ± 8.3	287.9 ± 4.2
	14	333.5 6.0	342.1 ± 8.5	312.7 ± 8.9	326.3 ± 5.4
	17	358.0 ± 6.9	369.2 ± 9.1	338.2 ± 10.3	349.8 ± 5.6
	20	402.9 ± 8.8	410.1 ± 9.7	377.6 ± 11.1	393.3 ± 7.6
	0-20	139.3 ± 5.1	135.6 ± 2.4	121.4 ± 6.4	136.0 ± 6.5

Data represent Mean ± S.E.

Table 21 Cesarean section findings of F1 offspring

Dose (mg/kg)		Control	1	3	10
No. of litters examined		8	9	9	9
No. of corpora lutea	Total	119	132	127	134
	Mean ± S.E.	14.9 ± 0.44	14.7 ± 0.44	14.1 ± 0.54	14.9 ± 0.45
No. of implantations	Total	117	130	118	123
	Mean ± S.E.	14.6 ± 0.56	14.4 ± 0.58	13.1 ± 0.42	13.7 ± 1.00
Implantation ratio		117/119	130/132	118/127	123/134
	Litter Mean (%)	98.2	98.3	93.3	91.0
No. of resorptions and dead fetuses	Total	8	8	6	5
	Litter Mean (%)	6.7	6.0	5.2	4.2
No. of live fetuses	Total	109	122	112	118
	Mean ± S.E.	13.6 ± 0.63	13.6 ± 0.58	12.4 ± 0.56	13.1 ± 1.05
Sex ratio (male/female)		1.02 (55/54)	1.44 (72/50)	1.04 (57/55)	0.90 (56/62)
Fetal body weight (g)	Mean ± S.E.	3.49 ± 0.06	3.45 ± 0.05	3.42 ± 0.02	3.54 ± 0.05
No. of fetuses with external anomalies (%)		0	0	0	0

SEGMENT II REPRODUCTION IN RATS:

A) Method

36 F at 0, 1, 4, and 16 mg/kg/day, by gavage, days 7-17 of gestation. (Day of confirmed copulation = day 0 of gestation.).

(Doses were said to be chosen based on a preliminary study in pregnant rats showing miosis at 5 mg/kg +, decreased weight gain, fasciculation, decreased spontaneous activity, and lacrimation at 10 mg/kg +, decreased food consumption at 20 mg/kg +, and lethality at 30 mg/kg; it was said that " no lethal effects on embryos and no teratogenic effects on the fetuses" were seen at these doses).

24 F/group had c-section on day 20 of gestation for fetal exam. (1/2 of fetuses were examined in head, thoracic, and abdominal regions using free hand razor method of Wilson and microscopic dissecting method of Nishimura. The remaining fetuses were examined for skeletal effects by the method of Dawson). The remaining dams (12/group) were allowed to deliver n[illegible]ly; F_1 weights, survival, developmental milestones, and postwea[illegible]g behavic[illegible]sts and reproductive performance were evaluated.

Strain:

Drug lot#: 88020201

B) Results

1) Observed signs in Fo

a) Miosis seen ~ 1/4 of MD and most HD

b) Fasciculation of limbs in most HD

c) Salivation and lacrimation in a few HD.

2) Fo mortality

None

3) Fo bodyweight

Decreased gain at MD and HD during the dosing period (mean weight on day 17 of gestation approx. 96 and 93% of control at MD and HD, resp.); increased gain in these groups during lactation period such that all groups had similar weight by end of lactation period. (See attached table).

4) To food consumption

Changes generally paralleled above bodyweight changes. Overall consumption during dosing period ~ 90 and 85% of control at MD and HD, resp. (See attached tables).

5) Results in dams sacrificed day 20 of gestation

Parameters measured and results obtained shown in tables "7", "11", and "12".

Fetal weight and placental weight were slightly decreased at HD. There were no effects on numbers of resorbed, dead, or live fetuses. (Table "7").

Fetal exams were said to show no drug-related effects; however, as shown in table 11, 5/159 (3.1%) fetuses at HD and 1/160 (0.6%) in controls had a ventricular septal defect. (The 5 at HD were seen in 5 separate litters; i.e. 21% of litters affected at HD vs. 4% in controls). (Ventricular septal defect was also seen in 1/165 MD fetuses). The report states that the incidence was within the spontaneously-occurring range for this strain of rats, but no data were presented. (See "Summary" section for further discussion). (Also note that visceral exam of the small number of stillborn and dying pups among dams allowed to deliver naturally showed a ventricular septal defect [+ other cardiovascular abnormalities] in 1 MD stillborn pup (Tables 16 and 17]).

6) Results in dams allowed to deliver naturally

Parameters measured and results obtained shown in tables "9" and"13" - "34".

The only possibly drug-related effect among the many parameters measured was an increase in open field ambulation among male pups at all doses at 5 weeks of age. (Table 20). There were no drug effects on other developmental, behavioral, or reproductive parameters among F_1 pups, or on F_1 pup weights and survival.

Table 1 Teratological study in rats treated orally with E2020

Clinical signs in F0 dams during the gestation period

Dose mg/kg	Symptoms	Administration 0-6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22a)
0	No. of dams	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	12	0
	No. of dams observed b)	36	31	32	31	23	31	24	24	28	24	29	23	36	36	36	12	0
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	No. of dams	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	12	0
	No. of dams observed b)	36	31	32	32	21	32	34	34	29	22	31	21	36	36	36	12	0
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	No. of dams	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	12	0
	No. of dams observed b)	36	31	32	31	22	33	33	34	29	22	30	22	36	36	36	12	0
	No. of dams with abnormal findings	0	6	7	5	4	9	6	9	4	7	6	6	0	0	0	0	0
	Miosis	0	5	7	5	4	9	6	9	4	7	6	6	0	0	0	0	0
16	No. of dams	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	12	0
	No. of dams observed b)	36	31	33	31	22	32	33	34	29	23	29	22	36	36	36	12	0
	No. of dams with abnormal findings	0	31	33	31	22	32	33	34	29	23	28	22	0	0	0	0	0
	Miosis	0	28	30	27	14	22	25	18	22	12	19	13	0	0	0	0	0
	Fasciculation on the limbs	0	29	32	31	21	30	33	34	28	23	29	22	0	0	0	0	0
	Lacrimation	0	2	18	12	4	8	3	2	5	2	6	3	0	0	0	0	0
	Salivation	0	0	4	4	1	1	2	4	3	3	10	8	0	0	0	0	0

a): Day of gestation
b): No. of dams observed 2 hours after treatment during the administration period, and No. of dams observed in A.M. during the other period.
Not all dams were scheduled to have 2-hour observation (see section 9. 1)).
No abnormal clinical signs were seen at times other than 2 hours after dosing.

STUDY NO. : R-24.

Table 2 Teratological study in rats treated orally with E2020

Clinical signs in F0 dams during the lactation period

Dose mg/kg	Symptoms	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21a)
0	No. of dams	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	No. of dams with abnormal findings	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	Nodule (right inguinal region)	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1	No. of dams	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	No. of dams	12	12	12	12	12	12	12	12 b)	11	11	11	11	11	11	11	11	11	11	11	11	11	11
	No. of dams with abnormal findings	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Nipple immaturity	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	No. of dams	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a): Day of lactation
b): One dam was necropsied, because all pups died.

STUDY NO.:R-248

Table 3 Teratelegical study in rats treated orally with E2020

Body weights of F0 dams during the gestation period

Dose mg/kg		Administration 0	3	7	9	11	13	15	17	20a)	Body weight gains 7-17	17-20
0	No.	36	36	36	36	36	36	36	36	36	36	36
	Mean	243.1	257.0	272.7	285.1	293.8	304.1	313.9	336.0	378.7	62.3	43.7
	S.D.	10.9	12.0	13.7	15.2	15.8	17.8	19.5	20.0	22.2	9.3	6.4
1	No.	36	36	36	36	36	36	36	36	36	36	36
	Mean	243.1	256.6	272.8	281.5	293.8	304.0	313.8	336.6	378.4	61.8	43.8
	S.D.	12.3	13.5	14.7	15.9	15.9	16.4	16.4	19.0	24.7	8.9	8.7
4	No.	36	36	36	36	36	36	36	36	36	36	36
	Mean	242.8	256.3	271.9	276.3	285.8**	293.6**	301.5**	321.7**	365.3**	49.8**	43.5
	S.D.	11.7	12.4	12.5	13.2	11.8	11.8	12.0	14.4	16.5	11.5	6.6
16	No.	36	36	36	36	36	36	36	36	36	36	36
	Mean	243.1	255.8	270.7	256.8**	268.0**	279.3**	292.7**	311.6**	354.9**	40.9**	43.3
	S.D.	11.9	13.4	15.3	15.6	15.0	16.8	17.4	17.1	18.7	9.4	6.8

No.: No. of dams Unit : g

a) : Day of gestation

* : $p<0.05$; ** : $p<0.01$ (Significant difference from control)

STUDY NO. :R-24

Table 4 Teratological study in rats treated orally with E2020

Body weights of F0 dams during the lactation period

Dose mg/kg		0	4	7	11	14	17	21a)	Body weight gains 0 - 21
0	No.	12	12	12	12	12	12	12	12
	Mean	288.8	294.6	308.8	318.5	317.2	311.7	303.8	17.0
	S.D.	22.9	14.9	16.9	18.2	15.8	13.7	14.1	14.4
1	No.	12	12	12	12	12	12	12	12
	Mean	285.3	293.2	301.4	312.7	310.6	313.9	300.8	15.5
	S.D.	16.9	17.0	16.8	12.2	14.3	17.1	16.3	12.2
4	No.	12	12	12	11[b]	11	11	11	11
	Mean	283.8	288.9	301.8	319.5	318.0	320.1	308.5	23.8
	S.D.	12.8	12.1	10.7	7.8	11.8	11.8	11.6	10.6
16	No.	12	12	12	12	12	12	12	12
	Mean	272.6	281.4*	295.6	308.0	314.1	311.6	300.9	28.3*
	S.D.	15.8	15.2	19.3	19.8	15.3	20.3	16.7	9.2

No.: No. of dams Unit : g
a) : Day of lactation
b) : One dam was necropsied on day 7 of lactation, because all pups died.
* : p<0.05 (Significant difference from control)

STUDY NO. :R-248

Table 5 Teratological study in rats treated orally with E2020

Food consumption of F0 dams during the gestation period

Dose mg/kg		Administration 1	4	8	11	14	17	20a)	Mean±S.D. 1-4b)	8-17c)
0	No.	36	36	36	36	36	36	36	36	36
	Mean	18.9	23.9	25.2	26.3	26.0	28.1	24.8	21.7	26.6
	S.D.	2.4	2.6	2.8	2.9	3.1	2.7	4.5	2.2	2.3
1	No.	36	36	36	36	36	36	36	36	36
	Mean	19.4	23.3	24.4	26.3	26.0	27.7	24.7	21.6	26.3
	S.D.	3.1	3.0	2.3	2.4	2.2	3.2	4.2	2.7	2.0
4	No.	36	36	35	36	36	36	36	36	36
	Mean	19.1	21.6	22.7**	23.6**	23.4**	25.7**	23.1	21.6	24.1**
	S.D.	2.6	2.8	2.2	2.3	2.2	2.3	4.3	2.8	1.5
16	No.	36	36	36	36	36	36	36	36	36
	Mean	19.1	23.3	17.6**	21.3**	24.7	25.7**	21.3**	21.6	22.4**
	S.D.	3.3	2.8	3.1	2.8	3.1	3.0	3.5	2.7	1.8

No.: No. of dams Unit : g/rat/day
a) : Day of gestation
b) : Mean food consumption of F0 dams during the pre-administration period
c) : Mean food consumption of F0 dams during the administration period
** : p<0.01 (Significant difference from control)

STUDY NO.: R-24

Table 6 Teratological study in rats treated orally with E2020

Food consumption of F0 dams during the lactation period

Dose mg/kg		1	4	7	11	14	17	21a)	Mean±S.D.b)
0	No.	12	12	12	12	12	12	12	12
	Mean	23.6	35.8	42.5	54.0	51.5	53.2	53.3	45.1
	S.D.	4.9	4.2	6.1	4.6	4.2	6.8	5.9	3.1
1	No.	12	12	12	12	12	12	12	12
	Mean	23.4	36.9	42.6	51.0	52.0	58.1*	63.5	46.8
	S.D.	6.0	5.8	8.8	4.5	5.4	5.6	8.8	3.9
4	No.	12	12	12	11c)	11	11	11	11
	Mean	23.1	33.7	42.2	54.8	58.0**	58.5*	67.9	48.7*
	S.D.	5.8	7.1	6.9	4.5	2.7	4.2	6.6	2.9
16	No.	12	12	12	12	12	12	12	12
	Mean	25.0	38.8	46.3	54.3	55.3**	60.3*	66.8	48.9*
	S.D.	4.3	4.4	3.7	8.2	4.9	6.5	8.5	4.0

No.: No. of dams Unit : g/rat/day

a) : Day of lactation:

b) : Mean food consumption of F0 dams during the lactation period

c) : One dam was necropsied on day 7 of lactation, because all pups died.

* : p<0.05 ; ** : p<0.01 (Significant difference from control)

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Table 7 Teratological study in rats treated orally with E2020

Cesarean section data on F0 dams

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implantations	Implantation index	No. of resorbed or dead fetuses			Live fetuses						Placental weight(g)	No. of live fetuses with ext. mal. (%)h)
									No. of live fetuses			Sex ratio (M/F)e)	Fetal body weight(g)			
					x a)	Total (%)b)	Early c)	Late d)	Total	Male	Female		Male	Female		
0	24	Total	362	348	96.1	16(4.6)	14	2	332	173	159	1.09				0(0.0)
		Mean	15.1	14.5					13.8	7.2	6.6		3.97	3.74	0.47	
		S.D.	1.4	1.6					1.7	2.1	2.6		0.20	0.16	0.04	
1	24	Total	377	361	95.8	18(5.0)	18	0	343	174	169	1.03				1(0.3)i)
		Mean	15.7	15.0					14.3	7.3	7.0		3.98	3.77	0.45	
		S.D.	1.2	1.4					2.0	2.2	2.5		0.21	0.21	0.04	
4	24	Total	370	358	97.0	22(6.1)	21	1	337	184	153	1.20				0(0.0)
		Mean	15.4	15.0					14.0	7.7	6.4		3.95	3.72	0.48	
		S.D.	1.8	1.3					1.7	2.0	1.7		0.22	0.25	0.04	
16	24	Total	366	351	95.9	21(6.0)	18	3	330	174	156	1.12				1(0.3)i)
		Mean	15.3	14.6					13.8	7.3	6.5		3.80**	3.61*	0.44*	
		S.D.	1.8	2.5					2.5	1.9	2.5		0.23	0.20	0.04	

a): (No. of implantations / No. of corpora lutea) X 100
b): (No. of resorbed or dead fetuses / No. of implantations) X 100
c): Resorbed embryo and placental remnant
d): Early macerated fetus, late macerated fetus and dead fetus
e): No. of males / No. of females
f): No. of live fetuses with external malformations
h): (No. of live fetuses with external malformations / No. of live fetuses) X 100
i): Omphalocele
* : $p<0.05$; ** : $p<0.01$ (Significant difference from control)

Table 8 Teratological study in rats treated orally with E2020

Macroscopic findings and ovary weights of F0 dams at cesarean section

Dose (mg/kg)			0	1	4	16
No. of dams examined			24	24	24	24
No. of dams with abnormal findings			0	0	0	0
Ovary (mg)	Right	Mean	53.2	52.0	53.0	54.2
		S.D.	11.8	10.0	13.5	15.9
	Left	Mean	47.9	50.7	48.7	44.7
		S.D.	12.4	12.6	11.8	13.9

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Table 9 Teratological study in rats treated orally with E2020

Delivery data on F0 dams

Dose mg/kg		No. of pregnant females	No. of females a)	Delivery index % b)	Gestation period	No. of implantations	No. of stillbirth (%)c)	Live born: No. of live born Total	Male	Female	Sex ratio (Male/Female)	Ext. malf. d) (%)e)	Live birth index % f)
0	Total	12	12	100.0		177	2(1.2)	159	78	83	0.92	0(0.0)	89.8
	Mean				21.6	14.8		13.3	6.3	6.9			
	S.D.				0.5	1.5		1.4	1.9	1.6			
1	Total	12	12	100.0		170	1(0.6)	156	70	86	0.81	0(0.0)	91.8
	Mean				21.7	14.2		13.0	5.8	7.2			
	S.D.				0.5	1.1		1.7	2.1	2.1			
4	Total	12	12	100.0		155	2(1.4)	146	71	75	0.95	0(0.0)	94.2
	Mean				21.7	12.9*		12.2	5.9	6.3			
	S.D.				0.5	2.3		2.6	1.4	2.2			
16	Total	12	12	100.0		170	0(0.0)	159	81	78	1.04	0(0.0)	93.5
	Mean				21.7	14.2		13.3	6.8	6.5			
	S.D.				0.5	1.5		1.3	2.0	1.9			

a): No. of females delivered of live pup
b): (No. of females delivered of live pup / No. of pregnant females) × 100
c): (No. of stillborn pups / No. of stillborn and live born pups) × 100
d): No. of live born pups with external malformations
e): (No. of live born pups with external malformations / No. of live born pups) × 100
f): (No. of live born pups / No. of implantations) × 100
* : p<0.05 (Significant difference from control)

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Table 10 Teratological study in rats treated orally with E2020

Macroscopic findings in F0 dams at weaning

Dose (mg/kg)	0	1	4	16
No. of dams examined	12	12	11a:	12
No. of dams with abnormal findings (%)	1 (8.3)	0	0	0
Right inguinal region Subcutaneous nodule (ϕ3cm)	1 (8.3)	0	0	0

a): One dam was necropsied on day 7 of lactation, because all pups died.

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Table 11 Teratological study in rats treated orally with E2020

Visceral examination of live fetuses

Dose (mg/kg)	0	1	4	16
No. of dams examined	24	24	24	24
No. of dams with abnormal fetuses (%)	16 (66.7)	12 (50.0)	17 (70.8)	17 (70.8)
No. of fetuses examined	160	166	165	159
No. of fetuses with abnormality (%)	27(16.9)	22(13.3)	26(15.8)	41(25.8)
Abnormality (%)				
Thymic remnant in neck	24(15.0)	22(13.3)	23(13.9)	34(21.4)
Ventricular septal defect	1(0.6)	0(0.0)	1(0.6)	5(3.1)
Left umbilical artery	2(1.3)	0(0.0)	3(1.8)	3(1.9)

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Table 12 Teratological study in rats treated orally with E2020

Skeletal examination of live fetuses

Dose (mg/kg)	0	1	4	16
No. of fetuses examined	172	176	172	169b)
No. of fetuses with anomaly (%)	1(0.6)	0(0.0)	0(0.0)	0(0.0)
Fusion of exoccipital bone and cervical vertebral arch	1(0.6)a)	0(0.0)	0(0.0)	0(0.0)
No. of fetuses with variation (%)	20(11.6)	18(10.2)	13(7.6)	22(13.0)
Cervical rib	6(3.5)	3(1.7)	2(1.2)	4(2.4)
Shortened 13th rib	0(0.0)	0(0.6)	0(0.0)	2(1.2)
14th rib	13(7.6)	11(6.3)	8(4.7)	15(8.9)
Splitting of thoracic vertebral body	1(0.6)	2(1.1)	2(1.2)	0(0.0)
Splitting of sternebra	0(0.0)	2(1.1)	1(0.6)	0(0.0)
Sacralization of lumbar vertebra	0(0.0)	0(0.0)	0(0.0)	2(1.2)
Progress of ossification				
Reduced ossification of cervical vertebral arch	0(0.0)	0(0.0)	1(0.6)	0(0.0)
No. of ossified sternebrae (%)				
1st	172(100.0)	176(100.0)	172(100.0)	169(100.0)
2nd	172(100.0)	176(100.0)	171(99.4)	169(100.0)
3rd	172(100.0)	176(100.0)	172(100.0)	169(100.0)
4th	172(100.0)	176(100.0)	172(100.0)	169(100.0)
5th	159(92.4)	163(92.6)	165(95.9)	154(91.1)
6th	172(100.0)	175(99.4)	171(99.4)	169(100.0)
No. of ossified metacarpus [Mean±S.D.]				
Right	4.00±0.00	4.00±0.00	3.99±0.04	4.00±0.00
Left	4.00±0.00	4.00±0.00	3.99±0.04	4.00±0.00
No. of ossified metatarsus [Mean±S.D.]				
Right	5.00±0.00	4.99±0.03	4.9 0.06	4.97±0.14
Left	5.00±0.00	4.99±0.03	4.99 0.06	4.97±0.14
No. of ossified sacral and caudal vertebrae [Mean±S.D.]	9.69±0.44	9.93±0.36*	9.83±0.63	9.61±0.67

a): Combined with 14th rib and hypoplastic ossification of thoracic vertebral body
b): One fetus specimen lost
* : p<0.05 (Significant difference from control)

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Table 13 Teratological study in rats treated orally with E2020

Body weights of F1 males

Dose mg/kg		0	4	7	11	14	17	21	28	35	42	49	56	63	70a)
0	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	6.1	9.3	14.7	22.4	27.8	33.1	42.1	76.1	130.6	188.8	242.5	297.7	344.9	380.0
	S.D.	0.4	0.8	1.2	2.0	2.5	2.8	4.5	8.3	11.4	14.6	19.8	21.7	23.1	23.7
1	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	6.0	9.4	14.7	23.0	28.2	33.7	43.2	76.6	130.7	185.1	240.2	296.7	345.4	382.6
	S.D.	0.4	0.9	1.0	2.2	1.4	1.3	2.8	6.0	9.9	13.3	16.2	17.7	19.8	22.4
4	No.	12	12	11[b]	11	11	11	11	11	11	11	11	11	11	11
	Mean	6.0	9.0	14.5	22.2	28.0	33.5	42.6	74.9	129.0	184.7	240.9	296.5	341.7	376.2
	S.D.	0.4	1.6	1.0	1.4	1.2	1.3	2.1	5.0	9.0	12.6	14.3	19.4	20.5	22.6
16	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	5.9	8.9	14.4	22.2	28.0	33.6	42.6	76.4	130.2	184.6	240.5	298.0	341.6	376.4
	S.D.	0.4	0.7	1.1	1.7	1.9	1.9	3.6	5.9	8.9	11.4	16.2	17.3	17.9	20.0

No.: No. of rats Unit : g

a) : Day after birth ;

b) : One litter died within day 7 after birth.

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Table 14 Teratological study in rats treated orally with Z2020

Body weights of F1 females

Dose mg/kg		0	4	7	11	14	17	21	28	35	42	49	56	63	70a)
0	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	5.7	8.9	14.6	21.9	27.3	32.5	41.2	70.5	113.8	146.9	174.0	197.6	219.4	237.4
	S.D.	0.3	0.6	0.9	1.3	1.4	1.8	2.6	4.2	5.0	5.2	5.4	6.9	8.8	10.1
1	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	5.7	9.1	13.9	21.4	27.1	32.2	41.1	69.4	112.9	146.2	174.5	197.1	220.8	237.9
	S.D.	0.5	0.9	1.2	1.8	1.7	2.1	3.1	5.0	7.8	8.4	8.3	10.1	12.1	14.1
4	No.	12	12	11 b)	11	11	11	11	11	11	11	11	11	11	11
	Mean	5.6	8.6	14.0	21.5	27.1	32.5	41.3	70.4	113.1	146.5	175.2	199.6	221.8	238.0
	S.D.	0.5	1.5	1.3	1.5	1.4	1.3	2.4	4.0	6.2	6.6	9.5	11.7	14.7	15.6
16	No.	12	12	12	12	12	12	12	12	12	12	12	12	12	12
	Mean	5.6	8.5	13.7	21.3	27.0	32.0	41.8	69.9	112.5	144.8	172.8	196.2	219.8	235.3
	S.D.	0.4	0.8	1.1	1.8	2.1	2.6	3.9	5.6	8.0	7.2	10.5	12.7	14.1	15.4

No.: No. of dams Unit : g

a) : Day after birth

b) : One litter died within day 7 after birth.

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Table 15 Teratological study in rats treated orally with E2020

Viability index of pups

Dose mg/kg		No. of F0 dams	Before F1 culling: Day 0 Live number d)	Before F1 culling: Day 4 Live number d)	Before F1 culling: Survival index on day 4 after birth x a)	No. of F0 dams	After F1 culling: Day 4 Live number d)	After F1 culling: Day 21 Live number d)	After F1 culling: Weaning index on day 21 after birth x b)	No. of F0 dams	After F1 weaning: Day 21 Live number d)	After F1 weaning: Day 70 Live number d)	After F1 weaning: Viability index on day 70 after birth x c)
0	Total	12	159	159	100.0	12	96	95	99.0	12	95	95	100.0
	Mean		13.3	13.3			8.0	7.9			7.9	7.9	
	S.D.		1.4	1.4			0.0	0.3			0.3	0.3	
1	Total	12	156	156	100.0	12	96	96	100.0	12	96	96	100.0
	Mean		13.0	13.0			8.0	8.0			8.0	8.0	
	S.D.		1.7	1.7			0.0	0.0			0.0	0.0	
4	Total	12	146	136	92.5	12	93	88	94.6	11e)	88	88	100.0
	Mean		12.2	11.3			7.8	7.3			8.0	8.0	
	S.D.		2.6	3.0			0.9	2.3			0.0	0.0	
16	Total	12	159	157	98.7	12	96	95	99.0	12	95	95	100.0
	Mean		13.3	13.1			8.0	7.9			7.9	7.9	
	S.D.		1.3	1.1			0.0	0.3			0.3	0.3	

a): (No. of live pups on day 4 / No. of live born pups on day 0) X 100
b): (No. of live pups on day 21 / No. of live pups on day 4) X 100
c): (No. of live pups on day 70 / No. of live pups on day 21) X 100
d): No. of live pups
e): One litter died within day 7 after birth.

STUDY NO.:R-248

Table 16 Teratological study in rats treated orally with E2020

Visceral examination of stillborn pups

Dose (mg/kg)	0	1	4	16
No. of pups examined	2	1	2	0
No. of pups with abnormality (%)	0	0	1 (50.0)	0
Abnormality (%)				
Ventricular septal defect	0	0	1 (50.0)[a]	0
Absence of aortic arch	0	0	1 (50.0)[a]	0
Abnormal origin of right subclavian artery	0	0	1 (50.0)[a]	0

a : All three abnormalities were noted in a single animal.

STUDY NO.:R-248

Table 17 Teratological study in rats treated orally with E2020

Visceral examination of F1 pups that died during the lactation period

Dose (mg/kg)	0	1	4	16
No. of dead pups	1 a)	0	18 b)	3 c)
No. of pups examined	0	0	8	1
No. of pups with abnormality (%)	0	0	1 (12.5)	0
Abnormality (%)				
Thymic remnant in neck	0	0	1 (12.5)	0

a): One pup could not examined because of eating by mother.
b): 8 pups could not examined because of eating by mothers.
c): 2 pups could not examined because of eating by mothers.

STUDY NO. :R-248

Table 18 Teratological study in rats treated orally with E2020

External development of F1 pups

Dose	No. of dams	Pinna detachment		Appearance of abdominal hair		Eruption of lower incisor		Opening of eyelid		Descent of testis		Opening of vagina	
mg/kg		14	7	7	11	11	14	14	17	21	28	38	42 a)
0	12	159/159	96/ 96	36/ 96	96/ 96	37/ 93	96/ 96	0/ 96	89/ 96b)	44/ 45	45/ 45	43/ 50	50/ 50
		100.0	100.0	39.6	100.0	38.5	100.0	0.0	92.7	97.8	100.0	86.0	100.0
1	12	156/156	96/ 96	36/ 96	96/ 96	24/ 96	96/ 96	1/ 96	89/ 96c)	46/ 47	47/ 47	37/ 49	49/ 49
		100.0	100.0	38.5	100.0	25.0**	100.0	1.0	92.7	97.9	100.0	75.5	100.0
4	12	130/135	88/ 88	42/ 88	88/ 88	51/ 88	88/ 88	0/ 88	79/ 88c)	42/ 45	45/ 45	37/ 43	43/ 43
		96.3**	100.0	47.7	100.0	58.0**	100.0	0.0	89.8	93.3	100.0	86.0	100.0
16	12	157/157	96/ 96	47/ 96	95/ 95	43/ 95	95/ 95	0/ 95	94/ 95c)	44/ 47	47/ 47	40/ 48	48/ 48
		100.0	100.0	49.0	100.0	45.3	100.0	0.0	98.9	93.6	100.0	83.3	100.0

Upper: No. of pups developed / No. of pups observed
Lower: %
a): Day after birth
b): Three pups were developed on day 18 after birth, four pups on day 19 after birth.
c): All pups were developed on day 18 after birth.
* : $p<0.05$; ** : $p<0.01$ (Significant difference from control)

STUDY NO. :R-248

Table 19 Teratological study in rats treated orally with E2020

Functional examination of F1 pups at weaning

Dose mg/kg	No. of F0 dams	Righting reflex	Pupillary reflex	Placing reflex	Corneal reflex	Preyer's reflex
0	12	95/ 95	95/ 95	95/ 95	95/ 95	95/ 95
		100.0	100.0	100.0	100.0	100.0
1	12	96/ 96	96/ 96	96/ 96	96/ 96	96/ 96
		100.0	100.0	100.0	100.0	100.0
4	11	88/ 88	88/ 88	88/ 88	88/ 88	88/ 88
		100.0	100.0	100.0	100.0	100.0
16	12	95/ 95	95/ 95	95/ 95	95/ 95	95/ 95
		100.0	100.0	100.0	100.0	100.0

Upper: No. of pups with normal response / No. of pups examined
Lower: %
a): One litter died within day 7 after birth.

STUDY NO. :R-248

Table 10 Teratological study in rats treated orally with E2020

Open field test of F1 males at 5 weeks

Dose	No. of	1st test							2nd test a)						
mg/kg	F1	Latency (sec.)	Ambu-lation	Amb.c	Rear-ing	Groom-ing	Defe-cation	Urina-tion	Latency (sec.)	Ambu-lation	Amb.c	Rear-ing	Groom-ing	Defe-cation	Urina-tion
0	22	17.8	24.9	0.3	5.5	2.3	2.0	0.4	17.1	9.9	0.0	2.5	2.4	1.3	0.4
		36.9	23.7	0.9	5.1	2.0	1.8	0.5	36.7	15.0	0.0	4.1	2.4	1.6	0.5
1	23	8.5	42.3*	0.8	8.8	1.9	1.2	0.3	8.0	20.0	0.3	2.9	2.0	1.5	0.3
		10.6	24.3	0.9	4.1	1.5	1.3	0.6	7.7	25.3	0.9	3.6	1.9	1.9	0.4
4	21	12.2	32.9	0.4	8.2	1.8	1.3	0.5	16.7	25.3*	0.3	4.4	2.6	1.7	0.2
		13.7	23.5	0.7	7.1	1.6	1.5	1.0	38.3	26.2	0.6	5.5	2.6	2.0	0.6
16	23	6.6	53.7**	0.7	8.6	1.4	2.0	0.3	6.3	27.5*	0.6**	4.5	1.8	1.9	0.1
		7.2	33.3	1.0	7.2	1.0	1.9	0.6	4.6	28.7	0.9	4.7	1.6	2.6	0.5

Amb.c: Ambulation in center area
a): The second test was conducted on the day after the first test
* : $p<0.05$; ** : $p<0.01$ (Significant difference from control)

Mean
S.D.

STUDY NO. :R-248

Table 21 Teratological study in rats treated orally with E2020

Open field test of F1 females at 5 weeks

Dose	No. of	1st test							2nd test a)						
mg/kg	F1	Latency (sec.)	Ambulation	Amb.c	Rearing	Grooming	Defecation	Urination	Latency (sec.)	Ambulation	Amb.c	Rearing	Grooming	Defecation	Urination
0	24	28.3	42.8	0.5	7.8	1.6	0.2	0.4	16.2	28.2	0.7	4.8	2.3	0.3	0.1
		40.0	27.0	0.9	5.8	1.3	0.8	1.1	18.2	29.2	1.3	5.5	2.2	1.0	0.3
1	24	19.8	50.7	1.1	7.8	1.0	0.6	0.5	11.4	44.0	0.8	5.8	2.0	0.2	0.6
		21.1	30.8	1.5	6.1	0.9	1.4	1.1	12.2	35.4	1.2	4.9	1.7	0.6	0.2
4	20	25.1	34.7	0.6	5.6	1.6	0.3	0.2	16.7	41.6	1.[illegible]	6.9	1.4	0.3	0.2
		[illegible]	24.8	1.1	4.3	1.2	0.8	0.4	18.2	44.5	3.2	6.8	1.2	0.8	0.4
16	24	21.3	61.9	1.1	9.3	1.2	0.8	0.2	22.4	28.0	0.4	4.5	0.8**	0.5	0.1
		34.4	30.1	1.4	7.0	1.1	1.6	0.4	40.4	31.0	0.8	6.3	1.2	1.7	0.3

Amb.c: Ambulation in center area
a): The second test was conducted on the day after the first test
** : p<0.01 (Significant difference from control)

Mean
S.D.

STUDY NO.:R-248

Table 22 Teratological study in rats treated orally with E2020

Water-filled multiple maze test of F1 males at 7 weeks

Dose mg/kg		Elapsed time (sec.) 1st day Trial 1	1st day 2	1st day 3	2nd day 1	2nd day 2	2nd day 3	3rd day 1	3rd day 2	3rd day 3	4th day 1	4th day 2	4th day 3
0	No.	22	22	22	21	22	22	22	22	22	23	23	23
	Mean	19.5	10.0	8.8	78.0	52.0	38.8	28.7	21.5	18.0	19.0	18.6	16.6
	S.D.	4.8	4.8	1.7	33.0	24.0	19.9	15.7	14.8	8.5	7.5	8.3	4.8
1	No.	23	23	23	23	23	23	23	23	23	23	23	23
	Mean	21.0	9.5	7.0	83.7	46.4	27.7	26.7	22.4	17.0	17.8	17.9	17.7
	S.D.	9.2	4.3	2.5	48.7	19.4	15.2	10.7	12.8	3.7	4.9	7.6	7.4
4	No.	21	21	21	20	21	21	21	21	21	21	21	21
	Mean	20.1	8.3	8.1	81.2	56.2	33.8	28.8	16.2	16.0	16.0	14.0*	14.3
	S.D.	9.8	3.0	2.0	29.4	34.7	20.9	17.2	3.7	6.9	5.6	2.5	3.0
16	No.	23	23	23	22	22	23	23	23	23	23	23	23
	Mean	20.1	9.3	8.7	73.0	55.0	37.3	34.3	20.2	18.8	20.8	16.1	14.7
	S.D.	8.3	6.1	3.9	34.9	31.8	30.2	20.4	8.5	8.3	14.8	6.2	3.5

No.: No. of F1 males which could reach goal
1st day: Straight channel
* : $p<0.05$ (Significant difference from control)

STUDY NO.:R-248

Table 23 Teratological study in rats treated orally with E2020

Water-filled multiple maze test of F1 males at 7 weeks

Dose mg/kg		Counts of error								
		Trial 1	2nd day 2	3	3rd day 1	2	3	4th day 1	2	3
0	No.	21	22	22	22	22	22	22	22	22
	Mean	4.0	3.3	1.7	1.3	0.7	0.3	0.3	0.5	0.1
	S.D.	2.2	2.0	1.3	1.7	1.5	0.7	0.5	1.3	0.3
1	No.	23	23	23	23	23	23	23	23	23
	Mean	4.0	2.7	1.2	1.0	0.6	0.3	0.2	0.1	0.1
	S.D.	2.0	1.4	1.1	1.2	1.3	0.4	0.4	0.3	0.3
4	No.	20	21	21	21	21	21	21	21	21
	Mean	3.7	3.4	1.7	1.5	0.4	0.4	0.3	0.1	0.2
	S.D.	2.2	2.0	1.4	1.9	0.6	0.9	0.6	0.4	0.5
16	No.	22	22	23	23	23	23	23	23	23
	Mean	4.3	3.3	1.8	2.0	0.7	0.6	1.0	0.6	0.3
	S.D.	2.0	1.9	2.1	1.8	0.8	0.8	1.7	0.8	0.6

No.: No. of F1 males which could reach goal

STUDY NO.:R-248

Table 24 Teratological study in rats treated orally with E2020

Water-filled multiple maze test of F1 females at 7 weeks

Dose mg/kg		Elapsed time (sec.) 1st day Trial 1	2	3	2nd day 1	2	3	3rd day 1	2	3	4th day 1	2	3
0	No.	24	24	24	22	21	24	24	24	24	24	23	24
	Mean	19.3	7.8	5.8	85.5	66.3	31.7	35.7	22.3	19.2	27.4	19.8	17.2
	S.D.	6.7	4.8	1.5	39.3	37.0	16.2	26.6	9.0	13.4	16.0	8.7	7.6
1	No.	24	24	24	23	24	24	24	24	24	24	24	24
	Mean	20.0	9.0	6.2	80.9	51.9	27.9	31.4	19.4	17.5	19.5*	18.8	17.9
	S.D.	11.6	3.9	1.8	28.1	26.3	12.4	23.1	8.0	5.0	10.4	8.4	7.8
4	No.	20	20	20	20	19	20	20	20	20	20	20	20
	Mean	19.3	8.4	6.4	85.1	63.4	30.1	25.5	22.7	18.1	22.4	15.4*	14.6
	S.D.	9.8	3.8	3.4	45.0	28.0	19.9	13.0	11.7	9.4	14.7	4.6	4.3
16	No.	24	24	24	21	21	24	24	24	24	24	24	24
	Mean	22.1	8.7	6.3	73.7	49.4	42.9	32.8	27.0	19.3	31.3	18.4	18.8
	S.D.	12.8	4.2	2.5	45.5	28.6	32.3	18.5	15.7	5.8	29.7	8.0	9.4

No.: No. of F1 females which could reach goal
1st day: Straight channel
* : p<0.05 (Significant difference from control)

STUDY NO.:R-248

Table 25 Teratological study in rats treated orally with E2020

Water-filled multiple maze test of F1 females at 7 weeks

Dose mg/kg		Counts of error								
		2nd day			3rd day			4th day		
		Trial 1	2	3	1	2	3	1	2	3
0	No.	22	21	24	24	24	24	24	23	24
	Mean	3.8	4.0	2.0	2.1	1.0	0.3	1.3	0.7	0.1
	S.D.	1.8	2.1	1.7	2.4	1.2	0.7	1.9	0.9	0.3
1	No.	23	24	24	24	24	24	24	24	24
	Mean	3.4	3.1	1.5	1.8	0.8	0.5	0.5	0.4	0.3
	S.D.	1.8	2.4	1.4	2.4	1.0	0.7	1.0	0.8	0.9
4	No.	20	19	20	20	20	20	20	20	20
	Mean	5.1*	4.3	1.7	1.6	1.3	0.7	1.0	0.3	0.2
	S.D.	2.1	2.9	1.9	1.7	1.4	1.2	1.7	0.7	0.5
16	No.	21	21	24	24	24	24	24	24	24
	Mean	4.4	3.3	2.5	1.8	1.5	0.8*	1.8	0.5	0.3
	S.D.	2.5	2.0	1.9	1.7	1.9	0.9	3.1	1.1	0.8

No.: No. of F1 females which could reach goal
* : $p<0.05$ (Significant difference from control)

STUDY NO.:R-248

Table 26 Teratological study in rats treated orally with E2020

Macroscopic findings in F1 animals used in behavioral test

Dose (mg/kg)	0	1	4	16
Male				
No. of F1 animals examined	22	23	21	23
No. of F1 animals with abnormal findings (%)	0	1 (4.3)	0	0
Prostate and seminal vesicle Atrophy	0	1 (4.3)	0	0
Female				
No. of F1 animals examined	24	24	20	24
No. of F1 animals with abnormal findings	0	0	0	0

STUDY NO. :R-24

Table 27 Teratological study in rats treated orally with E2020

Macroscopic findings in F1 animals at 10–12 weeks
(Extra animals from behavior and reproduction tests)

Sex	Dose mg/kg	F1 number	Findings
Male	4	3135-05	Malocclusion
		3136-05	NAF
Female	0	1130-16	NAF
		1136-16	NAF
	1	2134-15	NAF
	4	3128-15	NAF

NAF: No abnormal findings

Table 28 Teratological study in rats treated orally with E2020

Mating and fertility of F1 animals

Dose mg/kg	Male: No. of males	Male: Days until copulation Mean±S.D.	Male: Copulation index (%) a)	Male: Insemination index (%) b)	Female: No. of females	Female: Days until copulation Mean±S.D.	Female: Copulation index (%) c)	Female: Fertility index (%) d)
0	23	3.1±1.3	19/23(82.6)	18/19(94.7)	24	3.1±1.3	20/24(83.3)	19/20(95.0)
1	24	3.1±1.8	22/24(91.7)	22/22(100.0)	24	3.1±1.8	22/24(91.7)	22/22(100.0)
4	22	3.3±2.3	21/22(95.5)	21/21(100.0)	22	3.3±2.3	21/22(95.5)	21/21(100.0)
16	24	3.2±1.8	19/24(79.2)	18/19(94.7)	24	3.2±1.8	19/24(79.2)	18/19(94.7)

a): (No. of males with confirmed copulation / No. of males mated) X 100
b): (No. of inseminated males / No. of males with confirmed copulation) X 100
c): (No. of females with confirmed copulation / No. of females mated) X 100
d): (No. of pregnant animals / No. of females with confirmed copulation) X 100

STUDY NO. :R-24

Table 29 Teratological study in rats treated orally with E2320

Body weights of F1 dams during the gestation period

Dose mg/kg		0	4	8	12	16	20a)
0	No.	19	19	19	19	19	19
	Mean	249.9	266.9	283.2	304.8	334.5	392.7
	S.D.	18.9	20.1	21.3	25.6	26.9	35.8
1	No.	22	22	22	22	22	22
	Mean	253.8	269.4	286.4	310.4	337.4	390.3
	S.D.	20.1	22.1	24.9	25.9	29.8	32.9
4	No.	21	21	21	21	21	21
	Mean	255.1	270.3	287.7	307.9	339.8	395.4
	S.D.	23.6	25.7	26.8	25.1	26.9	30.2
16	No.	18	18	18	18	18	18
	Mean	251.2	267.6	285.4	310.8	338.4	395.4
	S.D.	17.2	20.9	21.7	24.5	26.5	29.8

No.: No. of F1 dams
a) : Day of gestation

Unit : g

STUDY NO. R-2348

Table 38 Teratological study in rats treated orally with E2020

Cesarean section data on F1 dams

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implantations	Implantation index x a)	No. of resorbed or dead fetuses			Live fetuses						Placental weight(g)	No. of f) live fetuses with ext. mal. (%)h)
						Total (%)b)	Early c)	Late d)	No. of live fetuses			Sex ratio (M/F)e)	Fetal body weight(g)			
									Total	Male	Female		Male	Female		
0	19	Total	799	271	9[illegible]	8(3.0)	8	0	263	128	135	0.95				0(0.0)
		Mean	15.7	14.3					13.8	6.7	7.1		3.75	3.53	0.47	
		S.D.	2.2	[illegible].9					3.0	2.8	2.4		0.39	0.36	0.03	
6	22	Total	322	306	95.0	2[illegible](7.2)	22	0	284	139	145	0.96				0(0.0)
		Mean	14.6	13.9					12.9	6.3	6.6		3.74	3.56	0.45	
		S.D.	2.3	2.7					2.7	2.4	2.3		0.14	0.20	0.07	
4	21	Total	313	301	96.1	11(3.7)	10	1	290	144	146	0.99				0(0.0)
		Mean	14.9	14.3					13.8	6.9	7.0		3.89	3.71	0.47	
		S.D.	1.6	1.6					1.5	1.9	2.4		0.38	0.34	0.04	
16	18	Total	275	259	94.2	14(5.4)	14	0	245	116	129	0.90				0(0.0)
		Mean	15.3	14.4					13.8	6.4	7.2		3.86	3.67	0.47	
		S.D.	1.7	2.8					2.8	1.8	2.0		0.22	0.18	0.05	

a): (No. of implantations / No. of corpora lutea) x 100
b): (No. of resorbed or dead fetuses / No. of implantations) x 100
c): Resorbed embryo and placental remnant
d): Early macerated fetus, late macerated fetus and dead fetus
e): No. of males / No. of females
f): No. of live fetuses with external malformations
h): (No. of live fetuses with external malformations / No. of live fetuses) x 100

STUDY NO. :R-24

Table 31 Teratological study in rats treated orally with E2020

Macroscopic findings and ovary weights of F1 dams at cesarean section

Dose (mg/kg)			0	1	4	16
No. of F1 dams examined			19	22	21	16
No. of F1 dams with abnormal findings (%)			0	0	0	0
Ovary (mg)	Right	Mean	57.4	58.1	63.0	64.6
		S.D.	10.8	13.8	15.0	13.1
	Left	Mean	52.7	51.7	51.2	53.8
		S.D.	14.8	11.5	10.4	10.6

Table 32 Teratological study in rats treated orally with E2020

Macroscopic findings in F1 males used in reproductive performance test

Dose (mg/kg)	0	1	4	16
No. of F1 males examined	23	24	22	24
No. of F1 males with abnormal findings (%)	1 (4.3)	0	1 (4.5)	0
Testis and epididymis				
Softening and atrophy (Bilateral or unilateral)	1 (4.3)	0	1 (4.5)	0

Table 33 Teratological study in rats treated orally with E2020

Histopathological findings in not copulated and infertile F1 males

Dose (mg/kg)	0					1		4	16					
F1 number	1125 -02a)	1127 -02a)	1132 -02a)	1134 -02a)	1135 -02b)	2125 -01a)	2131 -02a)	3130 -01a)	4125 -01a)	4130 -01a)	4132 -02a)	4133 -02b)	4136 -01a)	4135 -02a)
Testis														
Atrophy of seminiferous tubules	-	-	-	+++	-	-	-	-	-	-	-	-	-	-
Epididymis														
Atrophy	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Seminal vesicle	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prostate		c)d)												c)
Interstitial cellular infiltration	-	+	-	-	-	-	-	-	-	-	-	-	-	++

a):No. of evaluated animal b):Infertile animal c):Lymphocyte d):Histiocyte

-:No remarkable changes +-:Slight +:Mild ++:Moderate +++:Severe

STUDY NO.:R-248

Table 34 Teratological study in rats treated orally with E2020

Histopathological findings in not copulated and infertile F1 females

Dose (mg/kg)	0					1		4	16					
F1 number	1126 -12a)	1128 -12a)	1133 -12a)	1135 -12a)	1136 -13b)	2128 -12a)	2132 -12a)	3131 -11a)	4126 -11a)	4131 -11a)	4133 -12a)	4134 -12b)	4136 -11a)	4136 -12a)
Ovary	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Uterus	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a): Not copulated animal b): Infertile animal
- : No remarkable changes

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SEGMENT II REPRODUCTION IN RABBITS:

A) Method

16 F at 0, 1, 3, and 10 mg/kg/day, by gavage, days 6-18 of gestation. (Day of confirmed copulation = day 0 of gestation).

(Doses were said to be chosen based on a preliminary study in pregnant rabbits showing decreased food consumption at 10 mg/kg +, decreased bodyweight, perianal staining, emaciation and abortions at 20 mg/kg +, and 1 death [total N not given] at 30 mg/kg).

Does had c-section on day 28 of gestation for fetal exam. Approx. 1/2 fetuses were examined for visceral anomalies by the methods of Nishimura and Wilson. Remaining fetuses examined for skeletal effects by the method of Dawson.

Strain: Japanese White rabbits (SPF)

Drug lot #: 88020201

B) Results

1) Observed signs in does

No drug effects

Abortion occurred in 1 control and 1 MD

2) Doe mortality

No drug effects

One HD was sacrificed in extremis day 16 of gestation "because of a lumbar vertebra dislocation due to technical problem."

3) Doe bodyweight

No statistically significant differences in bodyweight; however it is noted that weights at HD decreased very slightly over the first 3 days of dosing (i.e. days 6-8 of gestation) whereas weights in other groups increased very slightly during this time (see attached table "1").

4) Doe food consumption

Decreased at HD days 8 and 10 of gestation (mean ~ 85% of control). (See attached table "2").

5) Reproductive parameters

No adverse drug effect on post-implantation loss, number of live fetuses, fetal weight, external fetal anomalies, etc. (Table "3").

6) Fetal visceral and skeletal exams.
(See tables "4" and "5")

No clear drug effects. The number of ossified sternebrae was statistically significantly increased at HD but examination of individual animal data does not indicate a meaningful effect; no other group differences in ossification were seen.

Table 1 Teratological study in rabbits treated orally with E2020

Body weights of dams

Dose (mg/kg)		Administration														Body weight gains	
		0	3	6	8	10	12	14	16	18	20	22	24	26	28a)	6~18	20~28
0	No.	15	15	15	15	15	15	1	15	15	15	14[d]	14	14	14	15	14
	Mean	3.55	3.67	3.73	3.75	3.78	3.79	3.84	3.84	3.85	3.88	3.96	3.98	4.01	4.04	0.12	0.13
	S.D.	0.27	0.27	0.25	0.25	0.25	0.24	0.24	0.23	0.23	0.30	0.26	0.26	0.26	0.26	0.12	0.08
1	No.	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	3.57	3.68	3.73	3.76	3.80	3.84	3.89	3.91	3.93	3.98	4.00	4.07	4.10	4.15	0.20*	0.18
	S.D.	0.20	0.21	0.22	0.23	0.24	0.24	0.24	0.26	0.26	0.27	0.28	0.29	0.30	0.31	0.08	0.08
3	No.	16	16	16	16	16	16	16	16	16	15[c]	15	15	15	15	16	15
	Mean	3.56	3.69	3.72	3.77	3.76	3.81	3.85	3.89	3.90	3.95	4.00	4.05	4.11	4.13	0.17	0.18
	S.D.	0.17	0.18	0.15	0.15	0.17	0.17	0.16	0.17	0.17	0.19	0.19	0.20	0.22	0.25	0.08	0.17
10	No.	16	16	16	16	16	16	16	15[b]	15	15	15	15	15	15	15	15
	Mean	3.54	3.68	3.73	3.67	3.68	3.68	3.73	3.76	3.80	3.82	3.88	3.92	3.98	4.02	0.08	0.20*
	S.D.	0.15	0.15	0.18	0.15	0.15	0.16	0.17	0.19	0.19	0.19	0.21	0.20	0.20	0.22	0.12	0.10

No.: No. of dams Unit : kg
a) : Day of gestation
b) : One dam sacrificed on day 16 of gestation because of lumbar vertebral fracture
c) : One dam aborted on day 20 of gestation
d) : One dam aborted on day 22 of gestation
* : $p<0.05$ (Significant difference from control)

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Table 2 Teratological study in rabbits treated orally with E2020

Food consumption of dams

Dose (mg/kg)		Administration 3	6	8	10	12	14	16	18	20	22	24	26	28a)
0	No.	15	15	15	15	15	15	15	15	15	14[d]	14	14	14
	Mean	190.5	189.9	184.9	178.3	166.3	166.4	154.4	161.1	158.6	164.3	130.9	112.6	116.1
	S.D.	15.2	16.4	17.1	22.9	44.3	38.3	59.1	54.7	53.9	23.4	31.8	40.1	51.3
1	No.	15	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	192.3	192.8	187.9	184.1	183.9	182.8	174.7	185.5	182.2	178.9	162.9	125.9	118.4
	S.D.	14.8	16.3	21.0	20.8	25.5	25.1	33.9	17.9	24.3	32.2	36.0	38.8	39.0
3	No.	16	16	16	16	16	16	16	16	15[c]	15	15	15	15
	Mean	188.6	190.1	185.4	180.9	184.4	181.6	180.3	161.2	178.7	184.1	163.0	143.7	141.2
	S.D.	17.0	14.4	19.3	23.1	24.1	19.3	21.2	44.6	23.1	29.4	55.8	50.5	54.1
10	No.	16	16	16	16	16	16	15[b]	15	15	15	15	15	15
	Mean	185.1	188.7	156.0**	151.6**	163.6	148.9	139.7	155.8	151.1	152.3	140.4	121.2	119.3
	S.D.	19.4	16.1	35.9	27.0	24.3	33.9	43.7	34.8	32.0	39.7	38.9	24.2	42.8

No.: No. of dams Uint : g/rabbit/day

a) : Day of gestation

b) : One dam sacrificed on day 16 of gestation because of lumbar vertebral fracture

c) : One dam aborted on day 20 of gestation

d) : One dam aborted on day 22 of gestation

** : $p<0.01$ (Significant difference from control)

22 b

384

STUDY NO.:R-24

Table 3 Teratological study in rabbits treated orally with E2020

Cesarean section data

Dose (mg/kg)	No. of dams		No. of corpora lutea	No. of implantations	Implantation index (%)a)	No. of resorbed or dead fetuses			Live fetuses						Placental weight(g)	No. of f) live fetuses with ext. mal. (%)h)
						Total (%)b)	Early c)	Late d)	No. of live fetuses			Sex ratio	Fetal body weight(g)			
									Total	Male	Female	(M/Fe)e)	Male	Female		
0	14	Total	162	137		26	9	17	111	53	58	0.91				0
		Mean	11.6	9.8	85.9	17.9			7.9	3.8	4.1		37.50	37.92	3.36	0.0
		S.D.	2.7	2.5					2.4	1.8	1.9		5.00	5.46	0.58	
1	15	Total	173	144		18	6	12	126	53	73	0.73				0
		Mean	11.5	9.6	83.6	11.2			8.4	3.5	4.9		38.38	38.23	3.31	0.0
		S.D.	2.5	2.8					2.4	2.2	2.2		4.05	5.35	0.62	
3	15	Total	167	146		9	4	5	137	68	69	0.99				0
		Mean	11.1	9.7	86.4	8.1			9.1	4.5	4.6		38.63	37.86	3.52	0.0
		S.D.	2.2	3.2					3.3	2.0	2.4		6.23	5.96	0.89	
10	15	Total	184	160		13	6	7	147	79	68	1.16				0
		Mean	12.3	10.7	87.0	8.0			9.8	5.3	4.5		38.04	37.07	3.55	0.0
		S.D.	2.6	3.0					2.9	2.3	1.8		5.28	5.50	0.78	

a) : (No. of implantations / No. of corpora lutea) x 100
b) : (No. of resorbed or dead fetuses / No. of implantations) x 100
c) : Resorbed embryo and placental remnant
d) : Early macerated fetus, late macerated fetus and dead fetus
e) : No. of males / No. of females
f) : No. of live fetuses with external malformations
h) : (No. of live fetuses with external malformations / No. of live fetuses) x 100
Mean of values for each litter

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Table 4 Teratological study in rabbits treated orally with E2020

Visceral examination of live fetuses

Dose (mg/kg)	0	1	3	10
No. of fetuses examined	54	62	65	74
No. of fetuses with abnormality (%)	2(5.0)	2(2.4)	0(0.0)	2(1.9)
Abnormality				
Supernumerary coronary orifices	2(5.0)	2(2.4)	0(0.0)	0(0.0)
Hypoplasia of the lung and heart	0(0.0)	0(0.0)	0(0.0)	2(1.9)
Variation of the aortic arch a) (%)				
Type-1	33(57.6)	40(59.1)	38(52.4)	37(51.0)
Type-2	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Type-3	9(19.9)	14(28.2)	25(40.3)*	15(20.0)
Type-4	12(22.5)	8(12.7)	6(7.3)	20(27.1)

a) : Type-1 ; Right common carotid artery arising from brachiocephalic trunk (Normal type)
Type-2 ; Right subclavian artery arising from aorta and running behind the esophagus and trachea
Type-3 ; Small artery arising from bifurcation site of left common carotid artery and brachiocephalic trunk
Type-4 ; Left common carotid artery arising from aorta

% : Mean of % for each litter

* : $p<0.05$ (Significant difference from control)

STUDY NO. : R-247

Table 5 Teratological study in rabbits treated orally with E2020

Skeletal examination of live fetuses

Dose (mg/kg)	0	1	3	10
No. of fetuses examined	57	84	68	73
No. of fetuses with abnormality (%)	1(1.8)	2(1.7)	4(4.8)	1(1.1)
Abnormality (%)				
Fusion of sternebrae	0(0.0)	1(1.7)	0(0.0)	1(1.1)
Splitting of the rib	0(0.0)	0(0.0)	1(1.1)	0(0.0)
Multiple abnormalities of the thoracic vertebrae and ribs	0(0.0)	0(0.0)	1(1.1)	0(0.0)
Hypoplasia of caudal vertebra	1(1.8)	0(0.0)	2(2.3)	0(0.0)
No. of fetuses with variation (%)	1(1.4)	7(12.1)	0(9.0)	2(2.2)
Variation (%)				
Accessory sternebra	1(1.4)	2(3.5)	0(0.0)	2(2.2)
Splitting of sternebra	0(0.0)	3(5.2)	0(0.0)	0(0.0)
Splitting of the thoracic vertebral body	0(0.0)	2(3.0)	0(0.0)	0(0.0)
No. of fetuses with 13th rib (%)				
Right	3(10.4)	2(3.6)	0(0.0)	1(1.8)
Left	3(5.0)	4(5.8)	4(6.4)	4(5.1)
Bilateral	17(29.3)	29(41.7)	17(28.1)	30(43.8)
Total	23(42.8)	35(51.0)	21(33.6)	35(49.6)
Progress of ossification (Mean±S.D.)				
Sternebrae	5.56±0.44	5.83±0.23	5.82±0.21	5.87±0.28*
Forelimbs (right)				
Metacarpal phalanges	4.33±0.10	4.90±0.00	4.83±0.15	4.97±0.13
Proximal phalanges	4.97±0.07	4.97±0.00	4.90±0.00	4.90±0.00
Middle phalanges	3.83±0.25	3.95±0.10	3.97±0.00	3.88±0.16
Distal phalanges	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
Forelimbs (left)				
Metacarpal phalanges	4.96±0.00	4.90±0.00	4.83±0.15	4.97±0.13
Proximal phalanges	4.90±0.05	4.97±0.00	4.90±0.00	4.90±0.00
Middle phalanges	3.81±0.27	3.95±0.10	3.97±0.00	3.88±0.16
Distal phalanges	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
Hindlimbs (right)				
Metatarsal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Proximal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Middle phalanges	3.79±0.05	4.00±0.00	3.95±0.05	4.00±0.00
Distal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Hindlimbs (left)				
Metatarsal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Proximal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Middle phalanges	3.93±0.05	4.00±0.00	3.95±0.05	4.00±0.00
Distal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Sacral and caudal vertebrae	18.89±0.32	20.11±0.53	19.01±0.24	19.98±0.01

Mean of values for each litter
% : Mean of % for each litter
* : p<0.05 Significant difference from control

STUDY NO.:R-247

SEGMENT III REPRODUCTION IN RATS:

A) Method

24 F at 0, 1, 3, or 10 mg/kg/day, by gavage, from day 17 of gestation through day 20 PP.

F_1 weights, survival, developmental milestones, and postweaning behavioral test and reproductive performance were evaluated.

Strain: Sprague-Dawley (from Japan SLC Inc., Hamamatsu, Japan)

Drug lot #: 89120401

B) Results

1) Observed signs in Fo

(See attached table "3")

a) Miosis at MD and HD (with deceasing incidence over the study; not seen at MD at day 20 PP)

b) Slight fasciculations at HD (and in 1/18 MD on first day of dosing only)

2) Fo mortality

None

3) Fo bodyweight

Weights at HD were below control, becoming statistically significant at day 20 of gestation and remaining so throughout the treatment period. (Mean weight approx. 90-95% of control; see attached tables "4" and "5").

4) Fo food consumption

Decreased at HD over the entire treatment period. (Initially ~ 75% of control, increasing to ~ 90-95% of control by the end of treatment).

5) Reproductive parameters

(See attached tables for parameters measured and results obtained).

a) Increased stillborns at HD (6.4% vs. 0.6% in controls); liveborns slightly decreased. (See table "8").

b) Pup survival of liveborns through day 4 PP equivocally decreased at HD (78.7% vs. 95.7%; see table "8"). This was not statistically significant by the sponsor's analysis. Examination of the individual animal data (see "appendix 29" and "appendix 32", attached after table "8", for control and HD data, resp.) indicates a possible drug effect: total litter loss seen in 3/24 HD and 1/24 control; partial litter loss seen in 4/24 HD and 1/24 (with only one dead fetus) control. All of the dams with total litter loss were observed to be not lactating on day 1 or 2 PP.

c) The reasons for the increased stillbirths and decreased pup survival noted above are not clear. It was stated that pups which died on days 0 and 1 after birth were not necropsied. Other dead pups were said to have no microscopic abnormalities. (It is not stated how many pups were examined). Necropsies done in small number of animals at and after weaning showed no drug effects. There were no external anomalies seen among live pups (table "12").

As noted above, dams with total litter loss days 1-4 PP were noted to be not lactating although overall nursing behavior was said to be unaffected by drug (table "9").

d) Pup weight decreased at HD on days 7-21 PP (mean ~ 90-92% of control); slight, non-statistically significant decreases seen at earlier times. (Tables "13" and "14"). Among F_1 studied for reproductive capacity, weights at HD were below controls at 28 days PP; weights then remained below controls through day 70 PP but not statistically significantly (Tables "27" and "28").

Table 3 General signs at 2 to 3 hours after administration

Code / Study No	PMAG S91512	Study : Segment III Generation : F0	Species (Strain) : Rat (Slc:SD) Route : P.O.	Sex : Female

Dose (mg/kg)		Day of observation G17	L07	L20
0	No of animals examined	16	21	22
	Normal	16	21	22
1	No of animals examined	18	24	24
	Normal	18	24	24
3	No of animals examined	18	24	24
	Normal	12	19	24
	Miosis	6	5	0
	Fasciculation (+)	1	0	0
10	No. of dams examined	16	21	21
	Normal	1	4	3
	Miosis	15	10	7
	Fasciculation (+)	13	12	14

No : Number of dams examined
G : Gestating L: Lactating (+): Slight

Table 4 Body weight (g)

Code : BNAG
Study No : S91512
Study : Segment III
Generation : F0
Species (Strain) : Rat (Slc:SD)
Route : P.O.
Sex : Female

Dose (mg/kg)		Days of gestation 0	14	17	18	19	20	21	22	23	17-20
0	No	24	24	24	24	24	24	24	22	0	24
	Mean	231.8	289.5	311.1	323.5	334.9	347.5	354.0	263.8		36.4
	S.D.	16.7	20.9	20.6	21.2	21.4	22.9	24.7	32.9		6.3
1	No	24	24	24	24	24	24	24	23	0	24
	Mean	232.8	290.3	313.8	325.1	335.8	348.5	356.6	258.6		34.7
	S.D.	14.8	19.4	21.8	22.5	22.6	24.1	27.3	22.2		6.5
3	No	24	24	24	24	24	24	24	22	1	24
	Mean	233.1	292.6	315.1	322.7	337.9	350.5	358.5	256.0	239.0	35.4
	S.D.	14.4	17.3	18.3	22.0	21.2	21.8	21.5	16.1		5.8
10	No	24	24	24	24	24	24	24	24	2	24
	Mean	231.9	288.8	309.4	314.7	321.0	329.8* a	334.3* a	257.2	256.5	20.4**a
	S.D.	15.6	19.0	20.5	20.9	21.9	22.5	21.6	29.6	4.9	8.4

No : Number of dams examined
Significantly different from Control * $P < 0.05$, ** $P < 0.01$ a : Dunnett

Table 5 Body weight (g)

Code : BNAG
Study No : S91512
Study : SegmentIII
Generation : F0
Species(Strain) : Rat (Slc:SD)
Route : P.O.
Sex Female

Dose (mg/kg)		Days of lactation 1	4	7	10	14	17	21	1-21
0	No	24	23	23	23	23	23	23	23
	Mean	257.8	277.2	285.0	296.3	297.3	300.3	294.7	34.8
	S.D.	20.2	14.4	12.3	14.4	13.9	13.5	16.3	15.7
1	No	24	24	24	24	24	24	24	24
	Mean	259.7	273.7	282.8	292.1	296.8	298.5	294.9	35.2
	S.D.	21.8	19.2	17.6	18.8	19.8	19.3	20.1	9.6
3	No	24	24	24	24	24	24	24	24
	Mean	257.3	274.3	282.7	293.0	297.2	300.3	295.1	37.8
	S.D.	17.8	17.2	16.4	14.8	14.5	14.5	16.3	14.9
10	No.	22	21	21	21	21	21	21	21
	Mean	242.4** a	251.1**a	264.4**a	272.1**a	275.8**a	282.3**a	280.4* a	38.4
	S.D.	16.3	13.9	14.8	16.0	16.7	16.2	16.9	14.1

No. : Number of dams examined
Significantly different from Control * $P < 0.05$, ** $P < 0.01$ a : Dunnett

Table 6 Food intake (g/animal/day)

Code	: BNAG	Study	: SegmentIII	Species(Strain)	: Rat (Slc:SD)	
Study No	: S91512	Generation	: F0	Route	: P.O.	Sex : Female

Dose (mg/kg)		Days of gestation 17 ~20
0	No.	24
	Mean	19.7
	S.D.	2.0
1	No.	24
	Mean	19.4
	S.D.	2.3
3	No.	24
	Mean	19.4
	S.D.	2.8
10	No.	24
	Mean	14.6 **a
	S.D.	2.3

No. : Number of dams examined
Significantly different from Control * P < 0.05 , ** P < 0.01 a : Dunnett

Table 7 *Food intake (g/animal/day)*

Code / Study No	BNAG S91512	Study / Generation	Segment III F0	Species (Strain) / Route	Rat (Slc:SD) P.O.	Sex	Female

Dose (mg/kg)		Days of lactation 1 ~ 4	4 ~ 7	7 ~ 10	10 ~ 14	14 ~ 17	17 ~ 21	1 ~ 21
0	No	23	23	23	23	23	23	23
	Mean	30.9	37.1	44.8	52.2	53.3	61.1	279.4
	S.D	3.1	2.3	2.7	3.4	3.9	3.2	12.3
1	No	24	24	24	24	24	24	24
	Mean	30.7	36.9	45.0	53.4	54.4	62.7	283.0
	S.D	3.9	2.8	3.8	3.6	4.7	5.2	18.7
3	No	24	24	24	24	24	24	24
	Mean	29.0	36.7	44.2	50.2	54.5	60.5	275.0
	S.D	4.9	3.7	3.7	3.4	3.9	4.6	20.0
10	No	21	21	21	21	21	21	21
	Mean	22.6**a	33.2**c	40.4**c	46.2**c	50.4	57.1	250.0**c
	S.D	4.9	4.2	5.1	5.7	5.8	7.9	30.6

No : Number of dams examined
Significantly different from Control ** P < 0.01 a : Dunnett c : Dunnett-Ranking

Table 8 Delivery and nursing

Code : BNAG	Study : Segment III	Species(Strain) : Rat (Slc:SD)	
Study No. : S91512	Generation : F0	Route : P.O	Sex : Female

		Dose (mg/kg)	0	1	3	10
No of pregnant dams			24	24	24	24
No of dams with live newborns			24	24	24	24
Delivery index (%)			100.0	100.0	100.0	100.0
Duration of gestation(days)	Mean±S.D.		21.9±0.3	22.0±0.2	22.0±0.4	22.1±0.3
No of implantation sites	Total		348	339	343	329
	Mean±S.D.		14.5±1.3	14.1±1.6	14.3±1.5	13.7±2.3
No of newborns	Total		328	314	320	296
	Mean±S.D.		13.7±1.3	13.1±1.7	13.3±1.6	12.3±2.2
No of live newborns	Total		326	311	314	277
	Mean±S.D.		13.6±1.2	13.0±1.7	13.1±2.1	11.5±2.6 **c
Sex ratio (Males / Females)			0.98 (161/165)	0.97 (153/158)	1.03 (159/155)	1.16 (149/128)
No of stillborns (%)			2(0.6)	3(1.0)	6(1.9)	19(6.4) **e
Live birth index (%)			93.7	91.7	91.5	84.2 **e
No of live pups on day 4	Total		312	309	313	218
	Mean±S.D.		13.0±3.0	12.9±1.7	13.0±2.0	9.1±4.4 **c
Viability index (%)			95.7	99.4	99.7	78.7
No of nursing dams on day 4			23	24	24	21
No of nursing dams on day 21			23	24	24	21
Nursing index (%)			95.8	100.0	100.0	87.5

Delivery index : (No of dams with live newborns / No. of pregnant dams) ×100
Live birth index : (No of live newborns / No. of implantation sites) ×100
Nursing index : (No of nursing dams on day 21 / No. of dams with live newborns) ×100
Viability index : (No of live pups on day 4 / No. of live newborns) x 100
Significantly different from Control * P < 0.05, ** P < 0.01 c : Dunnett-Ranking e : Wilcoxon-Rank Sum

Appendix 29 Delivery and nursing

Code : BNAG Study : Segment III Species (Strain) : Rat (Slc:SD)
Study No. : S91512 Generation : F0 Route : P.O.

Dose (mg/kg)	Dam No.	Animal fate	Duration of gestation (days)	Implantation sites No.	Stillborns No. (%)	Days after birth 0 (M/F)	(Pre-cull) 4 (M/F)	(Post-cull) 4 (M/F)	7 (M/F)	14 (M/F)	21 (M/F)
0	00F01		21	13	0(0.0)	13(7/ 6)	13(7/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F02		22	15	1(6.7)	14(8/ 6)	14(8/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F03		22	16	0(0.0)	15(9/ 6)	15(9/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F04		22	16	0(0.0)	15(12/ 3)	15(12/ 3)	8(5/ 3)	8(5/ 3)	8(5/ 3)	8(5/ 3)
	00F05		22	15	0(0.0)	12(3/ 9)	12(3/ 9)	8(3/ 5)	8(3/ 5)	8(3/ 5)	8(3/ 5)
	00F06		22	14	0(0.0)	13(4/ 9)	13(4/ 9)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F07		22	17	0(0.0)	16(8/ 8)	16(8/ 8)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F08		22	14	0(0.0)	13(10/ 3)	13(10/ 3)	8(5/ 3)	8(5/ 3)	8(5/ 3)	8(5/ 3)
	00F09	NL(002)	22	13	0(0.0)	13(5/ 8)	0(0/ 0)				
	00F10		22	13	0(0.0)	13(6/ 7)	13(6/ 7)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F11		22	14	0(0.0)	13(7/ 6)	13(7/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F12		22	15	0(0.0)	13(7/ 6)	13(7/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F13		22	16	0(0.0)	14(7/ 7)	13(6/ 7)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F14		22	14	0(0.0)	13(8/ 5)	13(8/ 5)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F15		22	12	0(0.0)	12(5/ 7)	12(5/ 7)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F16		22	12	0(0.0)	12(5/ 7)	12(5/ 7)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F17		22	15	1(6.7)	14(4/10)	14(4/10)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F18		22	16	0(0.0)	16(12/ 4)	16(12/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F19		22	15	0(0.0)	16(6/ 9)	16(6/ 9)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F20		22	16	0(0.0)	15(6/ 9)	15(6/ 9)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F21		22	15	0(0.0)	14(9/ 5)	14(9/ 5)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F22		22	14	0(0.0)	12(5/ 7)	12(5/ 7)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	00F23		21	14	0(0.0)	13(2/11)	13(2/11)	8(2/ 6)	8(2/ 6)	8(2/ 6)	8(2/ 6)
	00F24		22	14	0(0.0)	13(6/ 7)	13(6/ 7)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	Total			348	2(0.6)	326 (161/165)	312 (155/157)	184 (91/ 93)	184 (91/ 93)	184 (91/ 93)	184 (91/ 93)
	Mean		21.9	14.5		13.6	13.0	8.0	8.0	8.0	8.0
	S.D.		0.3	1.3		1.2	3.0	0.0	0.0	0.0	0.0

NL( ) : Not lactating (day of lactation) M / F : Male / Female

Appendix 32 Delivery and nursing

Code : BNAG Study : Segment III Species (Strain) : Rat (Slc:SD)
Study No. : S91512 Generation : F0 Route : P.O.

Dose (mg/kg)	Dam No	Animal fate	Duration of gestation (days)	Implantation sites No.	Stillborns No. (%)	Days after birth 0 (M/F)	(Pre-cull) 4 (M/F)	(Post-cull) 4 (M/F)	7 (M/F)	14 (M/F)	21 (M/F)
10	03F01		22	12	0(0.0)	11(7/ 4)	11(7/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F02		22	15	3(21.4)	11(5/ 6)	11(5/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F03	NL(001)	22	15	2(13.3)	13(10/ 3)	0(0/ 0)				
	03F04		22	12	1(9.1)	10(5/ 5)	10(5/ 5)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F05		22	15	0(0.0)	14(9/ 5)	14(9/ 5)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F06	NL(002)	22	17	1(6.7)	14(6/ 8)	0(0/ 0)				
	03F07		22	16	0(0.0)	13(7/ 6)	13(7/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F08		22	13	1(8.3)	11(3/ 8)	11(3/ 8)	8(3/ 5)	8(3/ 5)	8(3/ 5)	8(3/ 5)
	03F09		22	12	0(0.0)	11(6/ 5)	11(6/ 5)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F10		23	6	3(50.0)	3(2/ 1)	3(2/ 1)	3(2/ 1)	3(2/ 1)	3(2/ 1)	3(2/ 1)
	03F11	NL(001)	22	13	2(18.2)	9(5/ 4)	0(0/ 0)				
	03F12		22	13	0(0.0)	12(4/ 8)	12(4/ 8)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F13		22	14	1(7.1)	13(7/ 6)	5(2/ 3)	5(2/ 3)	5(2/ 3)	5(2/ 3)	5(2/ 3)
	03F14		22	15	0(0.0)	12(6/ 6)	12(6/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F15		22	12	0(0.0)	11(8/ 3)	11(8/ 3)	8(5/ 3)	8(5/ 3)	8(5/ 3)	8(5/ 3)
	03F16		22	13	0(0.0)	12(6/ 6)	10(6/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F17		22	15	1(7.1)	13(4/ 9)	13(4/ 9)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F18		22	14	1(8.3)	11(5/ 6)	11(5/ 6)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F19		22	13	0(0.0)	12(8/ 4)	12(8/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F20		22	14	0(0.0)	11(6/ 5)	11(6/ 5)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F21		22	14	0(0.0)	14(7/ 7)	10(5/ 5)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	03F22		22	10	1(6.3)	15(9/ 6)	6(2/ 4)	6(2/ 4)	6(2/ 4)	6(2/ 4)	6(2/ 4)
	03F23		23	14	2(22.2)	7(7/ 0)	7(7/ 0)	7(7/ 0)	7(7/ 0)	7(7/ 0)	7(7/ 0)
	03F24		22	14	0(0.0)	14(7/ 7)	14(7/ 7)	8(4/ 4)	8(4/ 4)	8(4/ 4)	8(4/ 4)
	Total			329	19(6.4)	277 (149/128)	218 (114/104)	157 (81/ 76)	157 (81/ 76)	157 (81/ 76)	157 (81/ 76)
	Mean		22.1	13.7		11.5	9.1	7.5	7.5	7.5	7.5
	S.D.		0.3	2.3		2.6	4.4	1.3	1.3	1.3	1.3

NL( ) : Not lactating (day of lactation) M / F : Male / Female

Table 9 Nursing behavior

Code : BNAG	Study : Segment III	Species(Strain) : Rat (Slc:SD)			
Study No : S91512	Generation : F0	Route : P.O.		Sex : Female	
	Dose (mg/kg)	0	1	3	10
No of dams with live newborns		24	24	24	24
	Days after delivery				
Eating placenta (+)	1	24/24(100.0)	24/24(100.0)	24/24(100.0)	24/24(100.0)
Retrieving (+)	1	23/24(95.8)	24/24(100.0)	24/24(100.0)	22/22(100.0)
	2	23/23(100.0)	24/24(100.0)	24/24(100.0)	21/21(100.0)
	3	23/23(100.0)	23/24(95.8)	24/24(100.0)	21/21(100.0)
	4	23/23(100.0)	24/24(100.0)	24/24(100.0)	21/21(100.0)
Lactation (+)	1	23/24(95.8)	24/24(100.0)	24/24(100.0)	21/22(95.5)
	2	23/23(100.0)	24/24(100.0)	24/24(100.0)	21/21(100.0)
	3	23/23(100.0)	23/24(95.8)	24/24(100.0)	21/21(100.0)
	4	23/23(100.0)	24/24(100.0)	24/24(100.0)	20/21(95.2)
Cannibalism (-)	1	24/24(100.0)	22/24(91.7)	24/24(100.0)	21/24(87.5)
	2	24/24(100.0)	24/24(100.0)	24/24(100.0)	21/22(95.5)
	3	23/23(100.0)	24/24(100.0)	23/24(95.8)	21/21(100.0)
	4	23/23(100.0)	24/24(100.0)	24/24(100.0)	21/21(100.0)

Identified / Examined (%)

Table 10 Necropsy findings

Code : BNAG	Study : Segment III	Species (Strain) : Rat (Slc:SD)			
Study No : S91512	Generation : F0	Route : P.O.		Sex : Female	
	Dose (mg/kg)	0	1	3	10
No. of animals examined		24	24	24	24
Necropsy findings					
subcutaneous mass in the thoracic region		0	1	0	0
subcutaneous mass in the abdominal region		0	1	0	1
subcutaneous abscess nodule in the right axilla		0	0	0	1

Table 11 Survival rate of pups

Code : BNAG	Study : Segment III	Species(Strain) : Rat (Slc:SD)			
Study No : S91512	Generation : F1	Route : P.O.		Sex : Male and Female	
	Dose (mg/kg)	0	1	3	10
No of nursing dams on day 4		23	24	24	21
No of live pups					
on day 4 (after culling)	Total	184	192	191	157
	Mean±S.D.	8.0±0.0	8.0±0.0	8.0±0.2	7.5±1.3 * a
on day 7	Total	184	192	191	157
	Mean±S.D.	8.0±0.0	8.0±0.0	8.0±0.2	7.5±1.3 * a
on day 14	Total	184	192	191	157
	Mean±S.D.	8.0±0.0	8.0±0.0	8.0±0.2	7.5±1.3 * a
on day 21	Total	184	190	191	157
	Mean±S.D.	8.0±0.0	7.9±0.3	8.0±0.2	7.5±1.3
Weaning index (%)		100.0	99.0	100.0	100.0

Weaning index : (No. of live pups on day 21 / No. of live pups immediately after culling) x 100
Significantly different from Control * P < 0.05 a : Dunnett

Table 12 External anomalies of pups

Code : BNAG	Study : Segment III	Species(Strain) : Rat (Slc:SD)			
Study No : S91512	Generation : F1	Route : P.O.			Sex : Male and Female
Dose (mg/kg)	0	1	3	10	
During days 1 to 4 after birth					
No of dams examined	24	24	24	22	
No of pups examined	325	309	314	228	
No of pups with anomalies (%)	0(0.0)	0(0.0))	0(0.0)	0(0.0))	
During days 4 to 21 after birth					
No of dams examined	23	24	24	21	
No of pups examined	184	192	191	157	
No of pups with anomalies (%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	

Table 13 Body weight (g)

Code : BSAG Study : Segment III Species(Strain) : Rat (Slc:SD)
Study No : S91512 Generation : F1 Route : P.O. Sex : Male

Dose (mg/kg)		Days after birth 1	4	4C	7	14	21	1-4	4C-21
0	No.	24	23	23	23	23	23	23	23
	Mean	6.4	9.6	9.5	15.0	28.5	44.2	3.0	34.7
	S.D.	0.7	1.1	1.0	1.4	1.9	3.3	0.6	3.1
1	No.	24	24	24	24	24	24	24	24
	Mean	6.8	9.9	9.9	15.4	29.1	45.6	3.1	35.6
	S.D.	0.6	0.9	0.8	1.0	2.0	3.9	0.4	3.4
3	No.	23	23	24	24	24	24	23	24
	Mean	6.5	9.5	9.5	14.6	27.6	43.1	3.0	33.7
	S.D.	0.5	1.0	0.9	1.6	2.7	4.0	0.6	3.5
10	No.	20	19	21	21	21	21	19	21
	Mean	6.2	9.2	9.3	13.9 •a	26.1 ••a	41.5	3.0	32.2
	S.D.	0.7	1.0	1.0	1.7	2.9	4.5	0.5	3.8

No : Number of litters examined C : Post-culling
Significantly different from Control • P < 0.05 , •• P < 0.01 a : Dunnett

Table 14 Body weight (g)

Code : BHAG
Study No : S91512
Study : Segment III
Generation : F1
Species(Strain) : Rat (Slc:SD)
Route : P.O.
Sex : Female

Dose (mg/kg)		Days after birth 1	4	4C	7	14	21	1-4	4C-21
0	No.	24	23	23	23	23	23	23	23
	Mean	6.1	9.1	9.3	14.6	28.0	43.3	3.0	34.1
	S.D.	0.8	1.1	1.1	1.4	1.8	2.7	0.5	2.5
1	No.	23	22	24	24	24	24	22	24
	Mean	6.4	9.7	9.7	14.8	28.1	44.0	3.2	34.3
	S.D.	0.5	0.7	0.8	1.1	1.8	3.2	0.4	2.9
3	No.	23	23	24	24	24	24	23	24
	Mean	6.2	9.2	9.2	14.3	27.2	42.8	3.0	33.6
	S.D.	0.6	0.9	0.9	1.3	2.5	3.9	0.5	3.6
10	No.	19	18	20	20	20	20	18	20
	Mean	5.8	8.8	8.8	13.4 **a	25.2 **a	40.1 **a	3.0	31.3**a
	S.D.	0.8	1.1	1.0	1.6	2.5	3.5	0.8	2.9

No. : Number of litters examined C : Post-culling
Significantly different from Control * P < 0.05 , ** P < 0.01 a : Dunnett

Table 15 Morphological and behavioral development of pups

Code : BNAG	Study : Segment III	Species (Strain) : Rat (Slc:SD)			
Study No : S91512	Generation : F1	Route : P.O.		Sex : Male	
	Dose (mg/kg)	0	1	3	10
No of pups positive / examined (%)					
Pinna unfolding	(on day 4)	155/155 (100.0)	152/152 (100.0)	159/159 (100.0)	114/114 (100.0)
Palmar grasp reflex	(on day 5)	91/ 91 (100.0)	99/ 99 (100.0)	98/ 98 (100.0)	81/ 81 (100.0)
Incisor eruption	(on day 14)	91/ 91 (100.0)	99/ 99 (100.0)	98/ 98 (100.0)	81/ 81 (100.0)
Auditory startle	(on day 15)	91/ 91 (100.0)	99/ 99 (100.0)	98/ 98 (100.0)	81/ 81 (100.0)
Eyelid opening	(on day 18)	91/ 91 (100.0)	99/ 99 (100.0)	98/ 98 (100.0)	81/ 81 (100.0)
Testicular descent	(on day 25)	23/ 23 (100.0)	24/ 24 (100.0)	24/ 24 (100.0)	20/ 21 (95.2)

Table 16 Morphological and behavioral development of pups

Code : BMAG	Study : Segment III	Species (Strain) : Rat (Slc:SD)			
Study No. : S91512	Generation : F1	Route : P.O.			Sex : Female
	Dose (mg/kg)	0	1	3	10
No. of pups positive / examined (%)					
Pinna unfolding	(on day 4)	157/157 (100.0)	157/157 (100.0)	154/154 (100.0)	104/104 (100.0)
Palmar grasp reflex	(on day 5)	93/ 93 (100.0)	92/ 93 (98.9)	93/ 93 (100.0)	76/ 76 (100.0)
Incisor eruption	(on day 14)	93/ 93 (100.0)	93/ 93 (100.0)	93/ 93 (100.0)	76/ 76 (100.0)
Auditory startle	(on day 15)	93/ 93 (100.0)	93/ 93 (100.0)	93/ 93 (100.0)	75/ 76 (98.7)
Eyelid opening	(on day 18)	93/ 93 (100.0)	92/ 92 (100.0)	93/ 93 (100.0)	76/ 76 (100.0)
Vaginal opening	(on day 38)	21/ 23 (91.3)	23/ 24 (95.8)	23/ 24 (95.8)	19/ 20 (95.0)

Table 17 Organ weight

Code : BMAG
Study No. : S91512
Study : Segment III
Generation : F1
Species(Strain) : Rat (Slc:SD)
Route : P.O.
Sex : Male

Dose (mg/kg)	No. of males	Body weight (g)	Liver (g)	Kidney (g)	Brain (g)
0	22	44.38±3.92	1.635±0.151	0.476±0.036	1.440±0.039
			(0.369±0.025)	(0.108±0.008)	(0.327±0.034)
1	24	45.50±4.68	1.689±0.237	0.492±0.047	1.443±0.035
			(0.370±0.018)	(0.108±0.006)	(0.320±0.031)
3	24	42.62±4.62	1.573±0.233	0.457±0.057	1.429±0.062
			(0.368±0.021)	(0.107±0.005)	(0.338±0.029)
10	18	42.63±4.41	1.564±0.222	0.460±0.051	1.425±0.051
			(0.366±0.020)	(0.108±0.006)	(0.337±0.027)

Mean±S.D. ( ) : Relative organ weight (g organ / 10 g body weight)

Table 18 Organ weight

Code : BNAG
Study No : S91512
Study : Segment III
Generation : F1
Species(Strain) : Rat (Slc:SD)
Route : P.O.
Sex : Female

Dose (mg/kg)	No of females	Body weight (g)	Liver (g)	Kidney (g)	Brain (g)
0	23	43.85±3.20	1.635±0.179	0.501±0.046	1.390±0.027
			(0.373±0.028)	(0.115±0.009)	(0.318±0.023)
1	23	44.05±3.23	1.633±0.149	0.504±0.041	1.402±0.044
			(0.370±0.016)	(0.114±0.005)	(0.320±0.021)
3	23	43.85±3.59	1.652±0.164	0.494±0.049	1.395±0.049
			(0.376±0.017)	(0.112±0.005)	(0.319±0.019)
10	19	40.63±3.53 **	1.504±0.155 *	0.463±0.046 *	1.365±0.047
			(0.370±0.016)	(0.114±0.003)	(0.338±0.021) **

Mean±S.D. ( ) : Relative organ weight (g organ / 10 g body weight)
Significantly different from Control * $P < 0.05$, ** $P < 0.01$

Table 19 *Multiple behavioral test*

Code : BNAG	Study : Segment III	Species (Strain) : Rat (Sic:SD)	
Study No : S91512	Generation : F1	Route : P.O.	Sex : Male

Dose (mg/kg)	0	1	3	10
No of abnormal males / examined (%)				
Pupillary reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)
Preyer's reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)
Corneal reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)
Pinna reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)
Ipsilateral flexor reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)
Pain response	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)
Struggle response	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)
Grip strength	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)
Gait	0 / 23 (0.0)	0 / 24 (0.0)	0 / 24 (0.0)	0 / 21 (0.0)

Table 20 Multiple behavioral test

Code : BNAG | Study : Segment III | Species (Strain) : Rat (Slc:SD)
Study No : S91512 | Generation : F1 | Route : P.O. | Sex : Female

Dose (mg/kg)	0	1	3	10
No of abnormal females / examined (%)				
Pupillary reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)
Preyer's reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)
Corneal reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)
Pinna reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)
Ipsilateral flexor reflex	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)
Pain response	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)
Struggle response	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)
Grip strength	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)
Gait	0 / 23 (0.0)	0 / 24 (0.0)	0 / 23 (0.0)	0 / 19 (0.0)

Table 21 Open field test

Code : BMAG	Study : Segment III	Species (Strain) : Rat (Slc:SD)			
Study No : S91512	Generation : F1	Route : P.O.			Sex : Male and Female
	Dose (mg/kg)	0	1	3	10
No. of males examined		23	24	24	21
No. of lines crossed		14.0 ±12.1	18.0 ±10.4	19.9 ±10.1	20.1 ± 8.9
No. of rearings		8.6 ± 7.3	11.0 ± 5.8	10.5 ± 7.0	13.7 ± 7.3
No. of groomings		1.4 ± 2.0	1.7 ± 1.6	2.5 ± 2.1	2.3 ± 1.2
No. of defecations		1.3 ± 1.6	1.4 ± 1.4	0.8 ± 1.4	0.5 ± 1.1
No. of urinations		0.1 ± 0.3	0.5 ± 0.5	0.3 ± 0.4	0.1 ± 0.5
No. of females examined		23	24	23	19
No. of lines crossed		20.4 ± 9.3	24.8 ± 8.7	20.4 ±10.3	23.8 ± 7.1
No. of rearings		12.3 ± 5.8	16.8 ± 7.0	13.2 ± 6.6	17.3 ± 8.4
No. of groomings		2.0 ± 1.4	2.7 ± 2.1	2.7 ± 1.7	2.4 ± 1.6
No. of defecations		0.7 ± 1.3	0.6 ± 0.9	0.4 ± 0.8	0.3 ± 0.8
No. of urinations		0.2 ± 0.7	0.2 ± 0.7	0.2 ± 0.5	0.0 ± 0.0

Mean±S.D.

Table 22 Rotarod test

Code : 3NAG	Study : Segment III		Species (Strain) : Rat (Slc:SD)		
Study No. : S91512	Generation : F1		Route : P.O.		Sex : Male and Female
Dose (mg/kg)	0	1	3	10	
No of males examined	23	24	24	21	
No of falls	1.0 ± 1.5	0.6 ± 0.8	1.0 ± 1.1	1.1 ± 1.1	
No of females examined	23	24	23	19	
No of falls	0.7 ± 0.8	0.5 ± 1.0	0.5 ± 0.8	0.4 ± 0.7	

Mean ± S.D.

Table 23 Water multiple T-maze test

Code : BNAG	Study : Segment III	Species (Strain) : Rat (Slc:SD)			
Study No. : S91512	Generation : F1	Route : P.O.			Sex Male
	Dose (mg/kg)	0	1	3	10
No. of males examined		23	24	24	21
Straight-way trials	Time in second	11.2 ± 5.4	11.8 ± 4.9	9.0 ± 2.9	9.9 ± 6.4
Maze trials	Time in second				
	day 1	46.3 ± 22.3	54.2 ± 22.3	50.6 ± 18.8	50.4 ± 18.8
	day 2	26.3 ± 33.0	21.3 ± 10.8	19.8 ± 10.0	22.9 ± 17.5
	day 3	21.0 ± 19.7	14.7 ± 5.4	15.7 ± 3.6	13.9 ± 3.4
	No. of errors				
	day 1	2.9 ± 1.6	3.5 ± 1.9	3.3 ± 1.3	3.4 ± 1.5
	day 2	1.4 ± 1.9	1.1 ± 1.1	1.0 ± 1.4	1.2 ± 1.5
	day 3	0.8 ± 1.3	0.4 ± 0.6	0.4 ± 0.5	0.4 ± 0.4

Mean ± S.D.

Table 24 Water multiple T-maze test

Code : BNAG	Study : Segment III	Species (Strain) : Rat (Slc:SD)			
Study No : S91512	Generation : F1	Route : P.O.			Sex : Female
	Dose (mg/kg)	0	1	3	10
No of females examined		23	24	23	19
Straight-way trials	Time in second	11.6 ± 7.6	11.8 ± 7.2	10.2 ± 3.9	9.1 ± 1.7
Maze trials	Time in second				
	day 1	49.9 ± 27.6	52.1 ± 21.8	51.3 ± 26.1	42.8 ± 19.2
	day 2	20.4 ± 13.4	19.9 ± 7.2	26.7 ± 32.2	20.6 ± 8.1
	day 3	13.7 ± 2.7	15.6 ± 5.1	17.7 ± 23.3	15.2 ± 4.2
	No. of errors				
	day 1	3.4 ± 1.7	3.4 ± 1.7	3.8 ± 1.9	3.0 ± 1.7
	day 2	1.2 ± 1.2	1.2 ± 0.9	1.5 ± 1.5	1.4 ± 1.0
	day 3	0.6 ± 0.6	0.6 ± 0.5	0.5 ± 1.0	0.5 ± 0.5

Mean ± S.D.

Table 25 General signs

Code : BNAG Study : Segment III Species (Strain) : Rat (Slc:SD)
Study No : S91512 Generation : F1 (Reproduction) Route : P.O. Sex : Male

Dose (mg/kg)	Findings	Days after birth 28	35	42	49	56	63	70
0	No. of males	23	23	23	23	23	23	23
	Normal	23	23	23	23	23	23	23
1	No. of males	24	24	24	24	24	24	24
	Normal	24	24	23	23	23	23	23
	Enlarged anterior chamber	0	0	1	0	0	0	0
	Opacity of eyeball	0	0	0	1	1	1	1
3	No. of males	24	24	24	24	24	24	24
	Normal	24	24	24	24	24	24	24
10	No. of males	21	21	21	21	21	21	21
	Normal	21	21	21	21	21	21	21

Table 26 General signs

Code : BNAG Study : Segment III Species (Strain) : Rat (Slc:SD)
Study No : S91512 Generation : F1 (Reproduction) Route : P.O. Sex : Female

Dose (mg/kg)	Findings	Days after birth 28	35	42	49	56	63	70
0	No. of females	23	23	23	23	23	23	23
	Normal	23	23	23	23	23	23	23
1	No. of females	24	24	24	24	24	24	24
	Normal	24	24	24	24	24	24	24
3	No. of females	24	24	24	24	24	24	24
	Normal	24	24	24	24	24	24	24
10	No. of females	20	20	20	20	20	20	20
	Normal	20	20	20	20	20	20	20

Table 27 Body weight (g)

Code : BNAG
Study No : S91512

Study : Segment III
Generation: F1 (Reproduction)

Species (Strain) : Rat (Slc:SD)
Route : P.O.

Sex : Male

Dose (mg/kg)		Days after birth 28	35	42	49	56	63	70	28-70
0	No.	23	23	23	23	23	23	23	23
	Mean	83.8	137.0	190.8	247.7	304.5	347.1	381.2	297.3
	S.D.	5.8	9.6	14.6	19.0	20.1	22.0	24.3	22.9
1	No.	24	24	24	24	24	24	24	24
	Mean	83.4	135.3	189.3	245.8	300.4	340.6	373.2	289.8
	S.D.	5.7	9.1	11.9	15.6	19.0	20.8	24.6	20.9
3	No.	24	24	24	24	24	24	24	24
	Mean	80.8	133.7	188.8	246.3	302.5	346.3	382.7	302.0
	S.D.	6.5	9.8	11.6	16.1	16.3	17.8	18.9	15.2
10	No.	21	21	21	21	21	21	21	21
	Mean	78.1* a	129.9	182.7	237.5	292.8	336.1	369.3	291.2
	S.D.	8.7	13.3	17.0	21.8	24.5	27.7	30.8	25.5

No : Number of males
Significantly different from Control * $P < 0.05$ a : Dunnett

Table 28 Body weight (g)

Code : BNAG
Study No : S91512
Study : Segment III
Generation : F1 (Reproduction)
Species (Strain) : Rat (Sic:SD)
Route : P.O.
Sex : Female

Dose (mg/kg)		Days after birth 28	35	42	49	56	63	70	28-70
0	No.	23	23	23	23	23	23	23	23
	Mean	76.3	116.7	146.3	171.3	194.0	211.3	225.7	149 4
	S.D.	5.2	6.9	9.6	11.5	13.1	14.6	16.5	15 9
1	No	24	24	24	24	24	24	24	24
	Mean	76 5	116.5	148.3	174.7	196.?	213 3	228.0	151 5
	S D	4.5	7.2	8.8	13.0	15.3	16.6	19.2	18 3
3	No.	24	24	24	24	24	24	24	24
	Mean	75.4	116.6	147.8	176.7	201.1	219.8	235.1	159 8
	S.D.	7.1	10.1	12.4	15.2	17.4	18.5	21.2	16 9
10	No.	20	20	20	20	20	20	20	20
	Mean	71.4* a	110.7	141.0	166.4	189.9	206.6	221.4	150 0
	S.D.	6.6	8.4	10.1	12.0	14.7	16.8	17.4	15 1

No. : Number of females
Significantly different from Control * P < 0.05 a : Dunnett

Table 29 Reproductive performance

Code : BNAG	Study : Segment III			Species (Strain) : Rat (Slc:SD)	
Study No. : S91512	Generation : F1			Route : P.O	
	Dose (mg/kg)	0	1	3	10
Male fertility					
No. of males					
Paired		22	24	23	20
Mated		21	17	19	17
Impregnating		21	16	18	16
Mating index (%) a)		95.5	70.8	82.6	85.0
Fertility index (%) b)		100.0	94.1	94.7	94.1
Female fertility					
First mating					
No. of females					
paired		22	24	23	20
Mated		21	17	19	17
Pregnant		21	16	18	16
Mating index (%) c)		95.5	70.8	82.6	85.0
Fertility index (%) d)		100.0	94.1	94.7	94.1
Day of mating (Mean ± S.D.)		3.0±2.9	2.2±1.6	3.5±2.0	3.7±3.0
Second mating					
No. of females					
paired		1	7	4	2
Mated		0	6	2	2
Pregnant		0	6	2	2
Mating index (%) c)		0.0	85.7	50.0	100.0
Fertility index (%) d)			83.3	100.0	100.0
Day of mating (Mean ± S.D.)			5.2±2.6	6.5±3.5	1.5±0.7
Calculation in total					
No. of females					
paired		22	24	23	20
Mated		21	23	21	19
Pregnant		21	21	20	18
Mating index (%) c)		95.5	95.8	91.3	95.0
Fertility index (%) d)		100.0	91.3	95.2	94.7
Day of mating (Mean ± S.D.)		3.0±2.9	3.0±2.3	3.8±2.3	3.5±2.9

a) Mating index : (No. of males with successful mating / No. of males paired with females) x 100
b) Fertility index: (No. of males impregnating females / No. of males with successful mating) x 100
c) Mating index : (No. of females with successful mating / No. of females paired with males) x 100
d) Fertility index: (No. of pregnant females / No. of females with successful mating) x 100

Table 30 General signs

Code : BNAG
Study No : S91512
Study : Segment III
Generation : F1
Species (Strain) : Rat (Slc:SD)
Route : P.O.
Sex : Female

Dose (mg/kg)	Findings	Days of gestation 0	7	14	17	20
0	No. of dams	21	21	21	21	21
	Normal	21	21	21	21	21
1	No. of dams	23	23	23	23	23
	Normal	23	23	23	23	23
3	No. of dams	21	21	21	21	21
	Normal	21	21	21	21	21
10	No. of dams	19	19	19	19	19
	Normal	19	19	19	19	19

Table 31 Body weight (g)

Code : BNAG
Study No.: S91512
Study : Segment III
Generation: F1
Species(Strain) : Rat (Sic:SD)
Route : PO
Sex : female

Group No. / Dose (mg/kg)		Days of gestation 0	7	14	17	20	Gain 0-20
0	N.	21	21	21	21	21	21
	Mean	265.3	296.8	331.9	357.3	403.1	137.9
	S.D.	20.7	23.6	24.3	29.1	33.4	19.2
1	N.	21	21	21	21	21	21
	Mean	272.5	302.2	336.6	361.2	401.4	128.9
	S.D.	29.8	27.4	27.7	27.6	29.2	15.6
3	N.	20	20	20	20	20	20
	Mean	270.6	296.2	331.0	359.1	401.2	130.6
	S.D.	18.1	16.2	17.3	18.0	24.0	21.9
10	N.	18	18	18	18	18	18
	Mean	261.7	288.4	326.8	352.1	393.6	131.8
	S.D.	25.6	24.6	26.1	29.3	33.4	17.1

No. : Number of dams examined

Table 32 Caesarean section data

Code : BNAG	Study : Segment III		Species(Strain) : Rat (Slc:SD)		
Study No : S91512	Generation : F1		Route : P.O.		
	Dose (mg/kg)	0	1	3	10
No. of dams examined		21	21	20	18
No. of corpora lutea	Total	314	335	301	278
	Mean±S.D.	15.0±2.0	16.0±2.0	15.1±1.5	15.4±2.1
No. of implantations	Total	307	303	285	271
	Mean±S.D.	14.6±2.2	14.4±2.8	14.3±2.4	15.1±2.0
Implantation index (%)		97.8	90.4	94.7	97.5
No. of dams with all resorptions		0	0	0	0
No. of resorptions and dead fetuses (%)		19 (6.2)	31 (10.2)	12 (4.2)	21 (7.7)
Early resorptions (%)		17 (5.5)	21 (6.9)	11 (3.9)	21 (7.7)
Late resorptions (%)		2 (0.7)	10 (3.3)	1 (0.4)	0 (0.0)
Dead fetuses (%)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. live fetuses	Total	288	272	273	250
	Mean±S.D.	13.7±2.1	13.0± 2.9	13.7±2.7	13.9±2.4
Sex ratio (Male/Female)		0.83 (131/157)	0.93 (131/141)	1.12 (144/129)	0.94 (121/129)
Fetal weight (g)	Mean±S.D.	3.50±0.19	3.42±0.18	3.45±0.18	3.39±0.12

Implantation index : (No. of implantations / No. of corpora lutea) x 100
Significantly different from Control * P < 0.05 , ** P < 0.01

Table 33 External anomalies of F2 fetuses

Code : BMAG	Study : Segment III	Species (Strain) : Rat (Slc:SD)	
Study No. : S91512	Generation : F1	Route : P.O.	

Dose (mg/kg)	0	1	3	10
No. of dams examined	21	21	20	18
No. of fetuses examined	288	272	273	250
No. of fetuses with anomalies (%)	0(0.0)	0(0.0)	1(0.4)	0(0.0)
Microphthalmia	0(0.0)	0(0.0)	1(0.4)	0(0.0)

Table 34 Necropsy findings

Code : BNAG	Study : Segment III	Species (Strain) : Rat (Slc:SD)			
Study No. : S91512	Generation : F1	Route : P.O.		Sex : Male and Female	
	Dose (mg/kg)	0	1	3	10
No. of males examined		23	24	24	21
Necropsy findings					
unilaterally small testis		1	0	1	1
unilaterally small seminal vesicle		0	1	0	0
unilaterally small epididymis		0	0	0	1
unilateral enlargement of the renal pelvis		0	0	0	1
No. of females examined		23	24	24	20
Necropsy findings					
abnormalities		0	0	0	0

GENOTOXICITY

A) Ames Test

Pre-incubation method used. Rat liver S9 was phenobarbital and 5,6 - benzoflavone-induced.

Strains used: Salmonella TA 1535, TA 1537, TA 98, and TA 100, and E. coli WP2/uvr A. Preliminary toxicity study showed bacterial growth inhibition at 500-1000 *ug*/plate; 1000 *ug*/plate was the HD used in the mutagenicity assay. E2020 was not mutagenic.

B) Chromosomal Aberrations in Chinese Hamster Lung Cells in Culture

Two assay systems were used: (1) nonactivation assay, where cells were treated with test drug for 24 or 48 hours, and (2) activation assay, where cells were treated with test drug either in presence or absence of rat liver S9 (phenobarbital - and 5,6-benzoflavone-induced) for 6 hours, followed by incubation in fresh drug-free medium for 18 hours.

An initial cytotoxicity test showed 50% cell growth inhibition at drug concentrations of 25 *ug*/ml and 178 *ug*/ml in the nonactivation and activation assays, respectively. In the nonactivation chromosomal aberration assay, E2020 was negative over the concentration range tested (3.1 - 50 *ug*/ml; results attached). In the activation assay, chromosomal aberrations were increased in the presence of S9 at the 2 highest drug concentrations tested (180 and 270 *ug*/ml) and in the absence of S9 at the highest evaluable concentration (180 *ug*/ml). (Results attached). Aberrations consisted mainly of chromatid breaks and chromatid exchanges; a few instances of chromatid gaps and fragmentation were also seen. It was concluded that E 2020 was clastogenic in this assay, but only at cytotoxic concentrations (attached results indicate % cell survival).

C) Mouse Micronucleus Test

Doses = 2.5, 5, and 10 mg/kg, by gavage, either acute or daily for 4 days. (Preliminary dose-ranging study showed deaths at 20 mg/kg +). Sampling time = 24 hr. after the last dose. E 2020 was negative.

Table 2 Chro[illegible]al aberration of E2020 in chinese hamster cells (CHL)

– Direct method – (NONACTIVATION ASSAY – 24 hr.)

Test substance	Concentration (μg/ml)	Time of treatment (h)	No. of cells (×10⁴/plate)	Survival ratio [a] (%)	No. of metaphase examined	No. of polyploid cells	Incidence [b] (%)	Judgement [c]	Structural aberrations: Types [d] and numbers (cumulative) ctg	csg	ctb	csb	cte	cse	others	No. of cells with chromosome aberration (+g)	(−g)	Incidence [e] (%) (+g)	(−g)	Judgement [c]
Non-treatment control	–	24	47	107	100	0	0	–	0	0	0	0	0	0	0	0	0	0	0	–
					100	0			0	0	0	0	0	0	0	0	0			
Vehicle control (Saline)	–	24	44	100	100	0	0.5	–	0	0	1	0	0	0	0	1	1	0.5	0.5	–
					100	1			0	0	0	0	0	0	0	0	0			
E2020	3.1	24	37	84	100	0	0.5	–	0	0	0	0	0	0	0	0	0	0	0	–
					100	1			0	0	0	0	0	0	0	0	0			
	6.3	24	41	93	100	0	0	–	0	0	0	0	0	0	0	0	0	0	0	–
					100	0			0	0	0	0	0	0	0	0	0			
	12.5	24	27	61	100	0	0	–	0	0	0	0	0	0	0	0	0	0	0	–
					100	0			0	0	0	0	0	0	0	0	0			
	25	24	23	52	100	0	0	–	0	0	0	0	0	0	0	0	0	0	0	–
					100	0			0	0	0	0	0	0	0	0	0			
	50	24	19	43	MM	–	–		–	–	–	–	–	–	–	–	–	–	–	
					MM	–			–	–	–	–	–	–	–	–	–			
Mitomycin C	0.1	24	33	75	100	0	0	–	0	0	25	0	37	0	0	47	47	46.5	46.0	+
					100	0			1	0	21	0	35	0	0	46	45			

a) Non-treatment control or E2020 treated group / Vehicle control

b) (Polyploid cells / observed metaphase cells) × 100

c) Judged on the basis of incidence as : − : negative(less than 5.0%). ± : suspicious(more than 5.0% to less than 10.0%). + : positive(more than 10.0%)

d) ctg : chromatid gap. csg : chromosome gap. ctb : chromatid break. csb : chromosome break. cte : chromatid exchange. cse : chromosome exchange

e) (Cells with structural chromosome aberration / observed metaphase cells) × 100

(+g) : total aberrant cells including the gap. (−g) : total aberrant cells excluding the gap

MM : Almost no metaphases

STUDY NO. :9719

STUDY NO. 9719

Table 2 Chromosomal aberration of E2609 in chinese hamster cells (CHL)
- Direct method - (NONACTIVATION ASSAY) (96 hr)

Test substance	Concentration (μg/ml)	Time of treatment (h)	No. of cells (×10⁴/plate)	Survival[a] ratio (%)	No. of cells examined	No. of polyploid cells	Incidence (%)	Judgment[b]	Structural aberrations: Types[c] and numbers (Cumulative) csg	csb	csb	csb	cte	cse	cse	others	No. of cells with chromosome aberration (-g)	(-g)	Incidence[d] (%) (-g)	(-g)	Judgment[e]
Non-treatment control	-	48	131	92	100	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	-
					100	0			0	0	0	0	0	0	0	0	0				
Vehicle control (Saline)	-	48	133	100	100	2	1.0	-	0	0	0	0	0	0	0	0	0	0	0	0	-
					100	0			0	0	0	0	0	0	0	0	0				
E2609	3.1	48	118	97	100	1	0.5	-	0	0	0	0	0	0	0	0	0	0	0	0	-
					100	0			0	0	0	0	0	0	0	0	0				
	6.3	48	92	98	100	0	0.5	-	1	0	0	0	0	0	0	0	1	0	0.5	0	-
					100	1			0	0	0	0	0	0	0	0	0				
	12.5	48	73	98	100	1	0.5	-	0	0	0	0	0	0	0	0	0	0	0	0	-
					100	0			0	0	0	0	0	0	0	0	0				
	25	48	69	52	100	1	0.5	-	0	0	2	0	1	0	0	0	2	0	1.5	1.5	-
					100	0			0	0	1	0	0	0	0	0	1				
	50	48	33	25	80	-	-		-	-	-	-	-	-	-	-	-	-	-	-	
					80	-			-	-	-	-	-	-	-	-	-				
Mitomycin C	0.1	48	51	35	100	0	0	-	1	0	64	0	21	0	0	0	44	44	43.5	43.5	+
					100	0			0	0	48	0	26	0	3*	0	37	37			

a) Non-treatment control or E2609 treated group / Vehicle control
b) (Polyploid cells / observed metaphase cells)×100
c) Judged on the basis of incidence as : - : negativeness than 5.0%. ± : positiveness than 5.0% to less than 10.0%. + : positiveness than 10.0%
d) csg : chromatid gap. csb : chromosome gap. cte : chromatid break. csb : chromosome break. cte : chromatid exchange. cse : chromosome exchange
e) (Cells with structural chromosome aberration / observed metaphase cells)×100
(-g) : total aberrant cells including the gap. (+g) : total aberrant cells excluding the gap
NR : almost no metaphase
* : fragmentation

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Table 5 Chromosomal aberration of E2020 in chinese hamster cells (CHL)

Metabolic activation method (-S9)

Test substance	Concentration (μg/ml)	S9 Mix	No. of cells ($\times 10^4$/plate)	Survival ratio (%)	No. of cells in metaphase examined	No. of polyploid cells	Incidence (%)	Judgement	Structural aberrations: Types and numbers (cumulative)							No. of cells with chromosome aberration (-g)	No. of cells with chromosome aberration (-g)	Incidence (%) (-g)	Incidence (%) (-g)	Judgement
									csg	csg	csb	csb	cte	cse	others					
Non-treatment control	–	–	10	104	100	0	0	–	0	0	0	0	0	0	0	0	0	0.5	0.5	–
		–			100	0			0	0	1	0	0	0	0	1	1			
Vehicle control (Saline)	–	–	61	100	100	0	0	–	0	0	0	0	0	0	0	0	0	1.5	1.5	–
		–			100	0			0	0	1	0	1	0	0	1	1			
E2020	11.3	–	73	109	100	0	0.5	–	0	0	0	0	0	0	0	0	0	0.5	0.5	–
		–			100	1			0	0	1	0	0	0	0	1	1			
	22.5	–	72	107	100	0	0	–	0	0	0	0	0	0	0	0	0	1.5	1.5	–
		–			100	0			0	0	1	0	0	0	0	1	1			
	45	–	61	91	100	0	0.5	–	0	0	1	0	0	0	0	1	1	2.0	2.0	–
		–			100	1			0	0	1	0	0	0	0	1	1			
	90	–	54	81	100	0	0	–	0	0	0	0	1	0	0	1	1	1.0	1.0	–
		–			100	0			0	0	1	0	0	0	0	1	1			
	180	–	25	37	100	0	1.0	–	0	0	43	0	32	0	4*	54	54	53.0	53.0	+
		–			100	2			0	0	38	0	32	0	2*	32	32			
	270	–	0	0	Tox	–	–		–	–	–	–	–	–	–	–	–	–	–	
		–			Tox	–			–	–	–	–	–	–	–	–	–			
Diethyl-nitrosamine	1000	–	62	93	100	1	0.5	–	0	0	2	0	0	0	0	2	2	2.0	2.0	–
		–			100	0			2	0	1	0	0	0	0	4	2			

a) Non-treatment control or E2020 treated group / Vehicle control

b) (Polyploid cells / observed metaphase cells) × 100

c) Judged on the basis of incidence as : – : negative(less than 5.0%). ± : suspicious(more than 5.0% to less than 10.0%). + : positive(more than 10.0%)

d) csg : chromatid gap. csg : chromosome gap. csb : chromatid break. csb : chromosome break. cte : chromatid exchange. cse : chromosome exchange

e) (Cells with structural chromosome aberration / observed metaphase cells) × 100

(-g) : total aberrant cells including the gap. (-g) : total aberrant cells excluding the gap

Tox : Cytotoxic effects

* : fragmentation

STUDY NO. 9719

Table 4 Chromosomal aberration of E2020 in chinese hamster cells (CHL)

- Metabolic activation method - (4 59)

Test substance	Concentration (μg/ml)	S9 Mix	No. of cells (×10⁴/plate)	Survival[a] ratio (%)	No. of metaphase examined	No. of polyploid cells	Incidence[a] (%)	Judgement[a]	Structural aberrations: Types[d] and numbers (cumulative) ctg	csg	ctb	csb	cte	cse	others	No. of cells with chromosome aberration (+g)	(-g)	Incidence[a] (%) (+g)	(-g)	Judgement[a]
Non-treatment control	–	–	86	93	100	0	0	–	0	0	0	0	0	0	0	0	0	0.5	0.5	–
		–			100	0			0	0	1	0	1	0	0	1	1			
Non-treatment control (+S9Mix)	–	+	75	100	100	0	0	–	0	0	0	0	0	0	0	0	0	0	0	–
		+			100	0			0	0	0	0	0	0	0	0	0			
Vehicle control (Saline)	–	+	71	100	100	0	0	–	0	0	0	0	0	0	0	0	0	0	0	–
		+			100	0			0	0	0	0	0	0	0	0	0			
E2020	11.3	+	65	92	100	0	0	–	1	0	0	0	0	0	0	1	0	1.0	0.5	–
		+			100	0			0	0	0	0	1	0	0	1	1			
	22.5	+	71	100	100	0	0.5	–	0	0	0	0	0	0	0	0	0	0	0	–
		+			100	1			0	0	0	0	0	0	0	0	0			
	45	+	58	82	100	0	0.5	–	0	0	0	0	1	0	0	1	1	0.5	0.5	–
		+			100	1			0	0	0	0	0	0	0	0	0			
	90	+	54	78	100	0	0	–	1	0	0	0	0	0	0	1	0	1.0	0.5	–
		+			100	0			0	0	0	0	1	0	0	1	1			
	180	+	30	42	100	0	0	–	1	0	6	0	1	0	0	7	0	6.5	0.6	±
		+			100	0			0	0	0	0	1	0	0	0	0			
	270	+	15	21	100	0	1.0	–	2	0	23	0	34	0	2*	52	51	48.5	47.5	+
		+			100	1			1	0	25	0	25	0	0*	45	44			
Diaethylnitrosamine	1000	+	67	86	100	0	0	–	1	0	18	0	56	0	0	59	55	62.0	62.0	+
		+			100	0			0	0	29	0	57	0	0	65	65			

a) Non-treatment control or E2020 treated group / Vehicle control

b) (Polyploid cells / observed metaphase cells)×100

c) Judged on the basis of incidence as : – : negative(less than 5.0%). ± : suspicious(more than 5.0% to less than 10.0%). + : positive(more than 10.0%)

d) ctg : chromatid gap. csg : chromosome gap. ctb : chromatid break. csb : chromosome break. cte : chromatid exchange, cse : chromosome exchange

e) (Cells with structural chromosome aberration / observed metaphase cells)×100

(+g) : total aberrant cells including the gap. (-g) : total aberrant cells excluding the gap

* : fragmentation

STUDY NO. :9719

SUMMARY

A) Pharmacodynamics

The putative mechanism of action of E2020 in Alzheimer's Disease is inhibition of acetylcholinesterase and resultant elevation of brain acetylcholine (which is decreased in this disease; an effect which is thought to play a role in the memory deficit.). The enzyme inhibition by E2020 was shown to be dialyzable in rat brain, indicating reversibility. Kinetic analysis indicated non-competitive inhibition in mouse brain, although an analysis in electric eel enzyme led to a conclusion of a mixed-type inhibition. Two metabolites ("M-1" and "M-3" had similar potencies to the parent compound in inhibiting rat brain acetylcholinesterase in vitro (see "Pharmacodynamics" section).

E 2020 produced various observed signs and physiological effects thought to be indicative of increased peripheral and/or central cholinergic activity, e.g. miosis, fasciculation, increased gastric motility, EEG arousal. Changes in urinary electrolyte excretion (generally increases during first few hours post-dosing, sometimes followed by decreases such that little change seen over a 24 hr. period post-dosing) were seen in both pharmacodynamics and toxicity studies in rats; some of these effects could be attenuated by scopolamine suggesting cholinergic mediation. A pharmacodynamic study in rats showed E2020 elevated fasting blood glucose and decreased fasting glucose tolerance at 10 (but not 3) mg/kg p.o. (similar effects produced by tacrine). (No consistent effects on blood glucose were seen in the toxicity studies).

The cardiovascular workup of E2020 in animals was fairly minimal. A "pilot" study in 3 anesthetized dogs showed no pronounced effects up to 1 mg/kg i.v. (The rationale for the doses used was not clear; doses of 0.3 and 1 mg/kg were said to cause tremors). In a "pilot" study in conscious rabbits, done primarily to evaluate drug effects on EEG, it was stated that 1 of 3 rabbits given the highest dose (1 mg/kg i.v.) had a transient decrease in heart rate (rate at 1 min. post dose ~ 45% pre dose). No drug effects were seen on EKG in the 3 month dog toxicity study, although the time relative to dosing when it was measured was not stated.

E2020 was negative in antigenicity studies in guinea pigs.

It is noted that most of the pharmacodynamic studies were non-GLP.

B) ADME/PK

The sponsor's ADME/PK summary is included earlier in this review; spot-checking indicates it to be reasonably accurate. Briefly, in the species used for toxicity studies (rats and dogs), E2020 is well absorbed and extensively metabolized, with a substantial amount of metabolites excreted into feces via bile. Levels of parent drug and several metabolites were measured in plasma, excreta and selected tissues. (The in vitro AchE inhibition by several metabolites was studied as noted above). It was stated by the sponsor that "There was no unique metabolite identified in humans that was not present in rats or dogs."

Some accumulation of parent drug with increasing duration of dosing was seen in toxicity studies. The reason for this is not clear since plasma $T^{1/2}$ for parent drug was relatively short (3-6 hr.), at least after acute dosing. ($T^{1/2}$ for total label was substantially greater .)

C) Toxicity Studies

1) Rat

An acute toxicity and the following repeat-dose studies were performed:

a) 3 month gavage (12-20/sex at 0, 0.3, 1, 3, 10, 20 mg/kg)

b) 1 year gavage (40/sex at 0, 1, 3, 10 mg/kg; 10/sex/group sacrificed at 6 months)

The acute oral median lethal dose was approx. 35 mg/kg p.o.; toxic signs included salivation, miosis, lacrimation, reduced spontaneous movement, staggering gait, tremors, clonic convulsions, respiratory depression, reduced surface body temperature, and prone position. A few deaths were seen at 10 and 20 mg/kg in the 3 month study, attributed to gavage errors possibly secondary to increased cholinergic tone. No drug-related deaths were seen in the 1 year study (HD = 10 mg/kg). In the 3 month study miosis and fasciculations were seen transiently at 3 mg/kg; at higher dosesthese signs + salivation, lacrimation, and hypoactivity were seen; 1 mg/kg was a sign-free dose in this study. In the 1 year study miosis was seen at all doses (LD = 1 mg/kg) with decreasing incidence during the second half of the study; other signs seen, mainly of low incidence and primarily at HD (10 mg/kg), included fasciculations, anogenital staining, alopecia, tail bumps, and soft stool.

Bodyweight gain was decreased at 10-20 mg/kg; in the one year study final weight at HD (10 mg/kg) was 11% and 15% below control in M and F, respectively. There was no clear drug effect on food consumption in the 3 month study; consumption was difficult to assess in the 1 year study since results were expressed as grams consumed per kg bodyweight, although it may be inferred that consumption was decreased at HD during the first 1 weeks (and possibly also at later times in HD M).

Ophthalmoscopic exams showed no drug effects (aside from lacrimation and miosis). Hematologic exams showed no clear drug effects. A few slight/equivocal blood chemistry effects were seen which were not consistent between the 3 month and 1 year studies. (In the 3 month study a few rats across all doses had elevated [2-17X highest concurrent control] ALT and/or AST and/or LDH. This was not seen in the 1 year study. In a 3 month dietary rangefinding study, mean values for ALT [but not AST or LDH] were slightly [1.4x control] increased in MD F [30 mg/kg] and HD F [60 mg/kg] but no individual values were greater than 2x the highest concurrent control. In a 3 month dietary study in mice enzyme elevations were seen in 1 of 10 mice each at 30 and 90 [but not 60] mg/kg. There were no drug-related histopathological effects in liver in any of these studies). (Mean values for BUN were very slightly increased in HD M at 6 months [with a trend in LD M and MD M] and in HD F at termination in the 1 year study, and in HD M and MD

and HD F in the above-mentioned rat dietary rangefinding study. There were no large individual increases, no elevations in creatinine, and no drug-related renal histopathology in any of these studies).

Urinalysis exams showed rather large changes in electrolyte excretion in both the 3 month and 1 year studies, although the nature of the effects were somewhat different. In the 1 year study, at the 3 month time point, urine was collected in 2 periods: 0-4 hr. post dose and 4-24 hr. post dose. In the former period, excretion of Na, K, and Cl was markedly increased in M and to a lesser extent in F. This was primarily due to increased electrolyte concentrations although urine volume was occasionally increased. Some of these effects were seen at all doses in M and in MD and HD F. These effects were not seen during the 4-24 hr. time period. Overall 0-24 hr. electrolyte excretion was slightly increased in MD and HD M with no effect in F. At the 6 and 12 mont time points, only 24 hr. measurements were taken; concentrations of 1 or more electrolytes were slightly increased at HD, but urine volume was slightly decreased resulting in no effect on electrolyte excretion. In the 3 month study, urine was sampled (week 13) from 4-23 hours post-dosing only; there were generally dose-related decreases in electrolyte excretion (sometimes affecting the lowest dose of 0.3 mg/kg), due to decreased electrolyte concentration without change in urine volume. (As noted under the pharmacodynamic studies, an acute dose of E2020 was shown to increase excretion of Na and Cl 0-4 hrs. post dose, and to decrease excretion of Na and Cl 4-24 hrs. post dose, with no net change over the 24 hr. period. [Effects on K were smaller/equivocal]. [In contrast to the results in the toxicity studies, these effects appeared to be due primarily to changes in urine volume rather than on electrolyte concentrations]. [Tacrine produced similar effects (measured 0-4 hr. post-dose) in this study. Also, the effects of E2020 on Na and Cl excretion (but not on K) were attenuated by scopolamine, suggesting cholinergic mediation]).

Salivary gland weights were increased in both studies at 10 mg/kg and above; this was associated with histological change (acinar cell hypertrophy, which was also seen at 3 mg/kg) in the 3 month study only. In the 3 month study, mucosal edema of the forestomach was seen at 10-20 mg/kg (with reduced effect after recovery period) and 1 of 12 rats at 20 mg/kg had forestomach erosion. These effects were not reported in the one year study. (In the acute toxicity study, rats which died had petechial hemorrhages in the glandular mucosa of the stomach along with "test substance-like residue" in the stomach). The incidence of slight hypertrophy of the pars intermedia of the pituitary was increased at HD (20 mg/kg) in the 3 month study but not in the 1 year study (HD 10 mg/kg).

Plasma drug levels measured in the 1 year study were roughly proportional to dose, tended to be greater in F than in M, and increased with increased duration of dosing.

2) Dog

An acute toxicity and the following repeat-dose studies were performed:

a) 3 month capsule (0, 0.3, 1, 3, 6, 8 mg/kg; see study for numbers of animals at each dose)

b) 1 year capsule (6/sex at 0, 0.6, 2, 5 mg/kg; 2/sex/group sacrificed at 6 months)

Oral doses of 8 mg/kg and above were lethal after 1 or 2 doses. Toxic signs prior to death included emesis, salivation fasciculations, tremors, staggering gait, and convulsions. Numerous lab test abnormalities were seen in these deaths, and post-mortem exam showed myocardial lesions (subendocardial hemorrhage, myocardial degeneration and necrosis) which were considered to be agonal/indirect effects, e.g. secondary to hypoxia. Toxic signs seen at lower doses included salivation, lacrimation, fasciculation, tremors, hyperactivity, and mucous stools. (Note that there were some differences between signs in the 3 month and 1 year studies, e.g. fasciculation and mucous stools were only reported in the former, and hyperactivity and lacrimation only in the latter. In the 3 month study the highest sign-free dose was 1 mg/kg, whereas in the 1 year study salivation was seen at all doses [LD = 0.6 mg/kg] [beginning prior to 3 months]). Food consumption was decreased during the first week at HD (5 mg/kg) in the 1 year study; bodyweights in HD F were slightly and equivocally less than controls throughout most of the study.

There were no clearly drug-related effects on ophthalmoscopic exam, EKG (done in 3 month study only; time relative to daily dose when measurements taken was not given) hematology, and blood chemistry. (As noted above, several lab test changes were seen with lethal doses which were considered to be secondary to toxic signs such as tremors or to be indirect/agonal effects. Also, very sporadic transaminase elevations were seen in a few drug-treated dogs at non-lethal doses which were not associated with unusual liver histopathology). Urinalysis in the 1 year study showed rather large dose-related decreases in urine volume and electrolyte excretion (but not urinary electrolyte concentrations) in MD and HD (and occasionally LD) M and in HD (and occasionally MD) F. The sponsor hypothesizes that these effects were due to (1) decreased water consumption (although, as noted earlier, the sponsor considered this to have occurred only in HD M, and even this was somewhat equivocal) and (2) salivation and lacrimation, which were drug-related signs in this study. (These urinalysis effects were not seen in the 3 month study, despite the fact that in some cases effects were seen by 3 months in the 1 year study at does which were below some doses used in the 3 month study.)

There were no clearly drug-related effects on organ pathology. (Again, some cardiac pathology was seen at lethal doses which was considered to be agonal/indirect). Submaxillary gland weight was equivocally increased in the 3 month study but equivocally decreased in the 1 year study.

Plasma levels of parent drug increased much greater than proportional to dose, and increased with increasing duration of treatment (particularly through the first month). There were no clear sex differences in plasma levels.

D) Genotoxicity Studies

E2020 was clastogenic (mainly chromatid breaks and exchanges; a few instances of chromatid gaps and fragmentation also seen) in Chinese Hamster lung cells in culture, but

only at concentrations which were cytotoxic. It was negative in an Ames Test and mouse micronucleus test (HD = 10 mg/kg p.o. acutely or daily for 4 days).

E) Reproduction Studies

The following reproduction studies were performed (mg/kg gavage doses in parentheses):

1) Segment I rat (1, 3, 10)
2) Segment II rat (1, 4, 16)
3) Segment II rabbit (1, 3, 10)
4) Segment III rat (1, 3, 10)

In the segment I study M were dosed from 63 days pre-mating through mating, and F were dosed from 14 days pre-mating through either day 7 of gestation (and sacrificed for c-section day 20 of gestation) or day 21 PP (natural delivery). There were no drug-related observed signs in the parents, which is unusual considering the signs seen in other rat reproduction and toxicity studies at similar doses. Bodyweight gains and food consumption were decreased in MD F and HD F (statistically significant in the latter only) at various times as noted earlier; there were no effects on these parameters in M. Estrous cycle was slightly prolonged at HD. There were no drug effects on fertility. There were no consistent effects on the various reproductive parameters measured; fetal weights were slightly increased among HD F sacrificed day 20 of gestation, but pup weights among HD F allowed to deliver naturally were similar to controls on day 1 PP and slightly less than controls (but not statistically significant) thereafter.

In the segment III study in rats, miosis was seen at MD and HD, and slight fasciculations were seen at HD (and 1/18 MD). Bodyweights and food consumption were below control at HD (mean weight ~ 90-95% of control). Stillborns were increased at HD (6.4% vs 0.6% in controls.). Number of liveborns was correspondingly slightly decreased at HD. Pup survival of liveborns through day 4 PP was equivocally decreased at HD (79% vs 96% in controls; not statistically significant according to the sponsor's analysis but individual data indicates possible effect as discussed earlier). Pup weights were decreased at HD from day 7 PP(and non-statistically significantly at earlier times). The reasons for the increased stillbirths and pup mortality are not clear. As noted earlier, morphologic exam of dead fetuses/pups appears to have been extremely limited. Also as noted earlier, dams with total litter loss days 1-4 PP (3/24 HD vs 1/24 controls) were noted to be not lactating, although overall nursing behavior was considered to have been unaffected by the drug. There were no drug effects among surviving pups on various developmental milestones, post-weaning behavioral tests, or reproductive performance.

In the segment II study in rats miosis was seen at MD (4mg/kg) and HD (16 mg/kg), and fasciculation, salivation, and lacrimation were seen at HD. Decreased weight gain and food consumption was seen at MD and HD during the treatment period (mean weight on day 17 of gestation ~ 96 and 93% of control, resp.). Among dams sacrificed on day 20 of gestation, fetal and placental weights were slightly decreased at HD. There were no drug effects on numbers of resorptions or dead fetuses. Fetal exams showed an incidence of

ventricular septal defect at HD of 5/159 (3.1%) fetuses and 5/24 (21%) litters vs control incidence of 1/160 (0.6%) fetuses 1/24 and (4%) litters. (Ventricular septal defect was also seen 1/165 fetuses at MD). The report states that these findings were within the spontaneously-occurring range for this strain of rats; a reference was cited but not included, and no further data were provided. (The reference cited has been submitted to another NDA. The page containing the data for ventricular septal defect for S1c : SD rats is attached. As shown, the mean incidence values across labs ranged from 0 [in 6 of the 11 labs listed] to 3.31%, with the highest value in a single study of 6.38%. In view of the wide range of values across labs, and the fact that the data was for studies done between 1980 and 1985, it would be much more useful to use historical incidence values obtained more recently from the lab performing the present study for comparison with the results of this study.).

In the segment II rat study 1/3 of the dams were allowed to deliver normally, with evaluation of F_1 weights, survival, developmental milestones, postweaning behavioral tests, and reproductive performance. The only possibly drug-related effect among the many parameters measured was an increase in open field ambulation among male pups at all doses at 5 weeks of age. In addition, it is noted that visceral exam done in the small number of stillborn and dying pups showed a ventricular septal defect (+ other cardiovascular abnormalities) in 1 MD stillborn.

In the segment II study in rabbits, there were no drug-related observed signs. Doe food consumption was deceased at HD (10 mg/kg) on days 8 and 10 of gestation (mean ~ 85% of control.) There was no statistically significant effect on doe bodyweight, although weights at HD decreased very slightly over the first 3 days of dosing (a time during which weights in other groups increased very slightly). (The doses used in this study were said to be based on a preliminary study in pregnant rabbits showing decreased food consumption at 10 mg/kg +, decreased bodyweight, perianal staining, emaciation, and abortions at 20 mg/kg +, and 1 death [total N not given] at 30 mg/kg). There were no noteworthy effects on post-implantation loss, fetal weight, or fetal exams.

Table 1-3B Continued

Laboratory	02	11	13	15	16	19	20	26	56	58	61
Strain	Stc SD	Stc SD	Stc SD	Stc SD	Stc SD	Stc SD	Stc SD	Stc SD	Stc SD	Stc SD	Stc SD
No. of fetuses examined	769	642	205	509	973	1352	1709	762	1052	2749	1228
No. of abnormal fetuses	94	13	0	123	20	189	21	7	58	180	246
%	12.22	1.96	0	24.17	2.06	13.98	1.23	0.92	5.51	6.55	20.03
Dilatation of cerebral ventricle					0.10 (0-1.02)						
Anencephalia										0.18 (0-2.03)	
Microphthalmia				0.20 (0-1.51)						0.11 (0-0.68)	
Anophthalmia								0.26 (0-1.20)	0.10 (0-1.32)	0.07 (0-0.96)	
Absence of optic nerve										0.11 (0-0.68)	
Tracheoesophageal communication										0.18 (0-1.06)	
Levocardia of heart				0.20 (0-1.61)							
Persistent atrioventricular canal										0.11 (0-1.06)	
Ventricular septal defect	0.13 (0-0.99)			0.20 (0-1.37)		1.11 (0-3.68)				1.31 (0-6.38)	0.24 (0-1.22)
Supernumerary coronary cusps				0.98 (0-2.70)						0.73 (0-1.87)	
Single coronary artery										0.04 (0-1.04)	
Hypoplasia of cardia				0.20 (0-1.52)							
Persistent truncus arteriosus	0.13 (0-1.02)										
Vascular ring										0.18 (0-1.36)	
Hypoplasia of pulmonary artery										0.11 (0-1.14)	
Sliding aorta				0.15 (0-1.00)						0.04 (0-1.04)	
Abnormal origin of right subclavian artery	0.13 (0-0.91)									0.07 (0-1.36)	0.16 (0-0.78)
Right subclavian artery arising from aortic arch										0.04 (0-1.25)	
Abnormal origin of left vertebral artery										0.13 (0-1.03)	
Abnormalities of pulmonary artery				0.39 (0-2.53)							
Persistent right azygos vein				0.39 (0-1.52)						0.22 (0-2.13)	1.63 (0-6.98)
Anomalous drainage of left azygos vein										0.33 (0-1.89)	
Anomalous course of azygos vein				0.20 (0-1.27)							
Hypoplasia of thyroid										0.36 (0-1.69)	
Thymic remnant in neck	11.70 (5.88-15.50)			10.41 (9.58-16.67)	0.31 (0-3.16)			1.24 (0-12.33)			14.50 (7.76-20.41)
Abnormal lobation of lung				4.91 (0-25.32)	0.10 (0-1.05)						8.80 (0-0.71)
Diaphragmatic hernia										0.04 (0-1.04)	
Persistent left umbilical artery				0.39 (0-1.27)					0.86 (0-7.89)	1.60 (0-3.87)	0.81 (0-3.40)
Abnormalities of liver				4.72 (0-11.39)							
Ectopic kidney											0.08 (0-0.70)
Dilatation of renal pelvis	0.13 (0-0.93)	1.96 (0-4.69)		0.39 (0-1.61)	1.44 (0-3.70)	12.87 (6.96-19.77)	1.23 (0-5.49)	0.66 (0-2.63)	3.33 (0-12.99)	0.22 (0-0.93)	1.30 (0-4.61)
Hypoplasia of kidney										0.04 (0-0.35)	
Absence of kidney										0.04 (0-1.04)	0.08 (0-0.65)
Hypoplasia of ureter										0.04 (0-1.03)	
Dilatation of ureter				0.39 (0-1.61)	0.10 (0-1.05)	3.62 (0-8.16)				0.11 (0-0.93)	3.01 (0-10.64)
Hypoplasia of adrenal										0.04 (0-0.93)	
Hypoplasia of intestine hern										0.07 (0-1.04)	
Absence of urinary bladder										0.07 (0-0.71)	

*, ( ) See table 1-3A.

EVALUATION:

This NDA is approvable.

Animal toxicity studies have gone up to 1 year in rats and dogs. Carcinogenicity studies in rats and mice are currently underway; it is current policy to allow these studies to be submitted post-marketing for this class of drugs. (Rat and mouse dietary rangefinding studies, and the proposed carcinogenicity protocols, were reviewed by me 3/1/94 and presented to the Carcinogenicity Assessment Committee [CAC] on 3/22/94; recommendations were transmitted to the sponsor in the letter of 4/7/94). The primary finding in the animal toxicity studies was the production of various cholinergic/CNS signs. Changes in urinary electrolyte excretion were seen in both rats and dogs but the nature of these changes varied across studies as discussed earlier (e.g. in some cases due to changes in urinary electrolyte concentration; in others to changes in urine volume). The sponsor hypothesizes that the changes in rats were cholinergically mediated since they were attenuated by scopolamine, and that the changes in dogs were due to decreased water consumption, salivation, and lacrimation. The were no consistent changes in plasma electrolyte concentrations. Sporadic elevations of serum transaminases and/or LDH were seen in some studies in rat, mouse, and dog, however these were too scattered to be considered to be drug-related within the resolving power of these studies, and were not associated with hepatic or other histopathology. Salivary gland weight was increased in both the 3 and 12 month rat studies; histologically salivary gland acinar cell hypertrophy were seen in the 3 month study only.

E2020 was clastogenic in Chinese Hamster lung cells in culture, but only at cytotoxic concentrations. It was negative in mouse micronucleus and Ames tests.

In a segment II reproduction study in rats an equivocal increase in the incidence of ventricular septal defect was seen at HD (16 mg/kg). The sponsor stated that the incidence was within the historical control range. The paper cited in support of this showed a wide range of values across laboratories, mostly quite low but in some cases above that seen at HD in the present study. In view of this wide range, and the fact that this historical data was only from studies done 1980-85, it would be more useful to have data obtained more recently from the lab performing the E2020 study; this information should be requested from the sponsor. (No teratological effects were seen in the segment II rabbit study.)

In the segment III rat study, the number of stillborns was increased, pup weights were decreased, and pup survival through day 4 PP was equivocally decreased, at HD (10 mg/kg). As discussed earlier, the cause of these effects (including the role of maternal toxicity) is not known. (In the segment I study, in which dams were treated from 14 days pre-mating through either day 7 of gestation or day 21 PP, there were no drug effects on stillbirths or pup mortality; weights of pups from HD dams allowed to deliver naturally became slightly less than control beyond day 1 PP but this was not statistically significant. The reason for this difference is not clear. The segment I and III studies were done by the same lab using the same strain, doses, and drug lot #. Decreased dam food consumption and weight gain were seen in both studies. Observed signs in dams were seen only in the segment III study, but these were not described as severe. One possibility is that the longer duration of dosing in the segment I study led to tolerance to some effect responsible for the increased stillbirths and pup mortality seen in the segment III study.). (In the segment II rat study, among F sacrificed day 20 of gestation, there were no drug effects on fetal deaths; fetal weight was

slightly decreased at HD [16 mg/kg]. Among F allowed to deliver, there were no drug effects on pup deaths, survival, or weights).

It is noted that no drug-related effects were seen in M in the segment I study, thus raising the question of whether this was an adequate test of male fertility. It is noted that at least some of the doses used in M did produce observed signs and/or decreased weight gain in general toxicity studies. (There were also no observed signs in F in the segment I study, in contrast to effects seen at similar doses in other reproduction and toxicity studies; although, in contrast to M, F in the segment I study showed decreased weight gain and food consumption).

We had previously suggested to the sponsor (e.g. meetings of 9/21/93 and 11/13/95) that additional ADME/PK data in animals would be useful particularly regarding the metabolic pattern in the species used for toxicity testing and pharmacologic activity of metabolites. Satisfactory data on these points were submitted with the NDA as reviewed herein.

RECOMMENDATIONS:

This NDA is approvable.

For the purpose of interpreting the results of the segment II reproduction study in rats, the sponsor should be requested to submit historical control incidence data for the finding of ventricular septal defect in the strain of rats used, preferably to include recent data from the lab which performed the E2020 study.*

Barry Rosloff

Barry N. Rosloff, Ph.D.

*Note added in proof: This was done by the CSO by phone on 6/11/96. **

cc:HFD-120\NDA20-690
\BRosloff
\GFitzgerald 7/2 7/96
\CSO Higgins
\Levin

rd\tcb\6\13\96
fd\tcb\6\25\96

n:\rosloff\e2020.wpd

** Note added in proof 7/11/96 - See attached

NOTE ADDED IN PROOF:

The requested historical control data were submitted by the sponsor on 6/27/96; see attached page. As indicated, the overall percent of fetuses with ventricular septal defect in the lab which performed the E-2020 study was 0.8% with a range of 0-2.9%. The analogous values for affected litters were 5.1% and 0-18.2%. As noted earlier, published values for mean fetal incidence ranged from 0-3.3% across labs with a range of 0-6.4% across individual studies. In light of these values, and the fact that no other teratogenic effects were apparent in the segment II studies in rats and rabbits, it cannot be concluded that E-2020 clearly increased the incidence of ventricular septal defect. (Incidence at HD in rat study was 3.1% of fetuses and 21% of litters). However, due to the finding of increased stillbirths and slightly decreased pup survival seen at HD in the segment III study, the pregnancy category in labeling should be "C". (The reason for these effects are not known; it would have been enlightening to have examined these dead/dying pups internally, e.g. for ventricular septal defect; however such exams were extremely limited as noted earlier.)

Historical control incidence data for "Ventricular septal defect (VSD)" in Slc:SD rats

<1985. 2 - 1990. 1>

Study Number	R-136*	R-148	R-218	R-220	R-248**	Total
Numbers of Dams	21	21	21	23	24	110
Numbers of observed fetuses	122	126	122	141	160	671
Numbers of fetuses with VSD (Dams with VSD)	1(1)	0	0	0	1(1)	2
Incidence of VSD (% of fetuses)	0.8	0.0	0.0	0.0	0.6	0.30
Incidence of VSD (% of dams)	4.8	0.0	0.0	0.0	4.2	1.8

* : This was the first teratology study in rats conducted at the Gotemba Laboratories.
**: R-248 (E2020)

<1990. 2 - 1995. 1>

Study Number	R-448	R-450	R-463	R-593	Total
Numbers of Dams	22	24	23	19	88
Numbers of observed fetuses	138	155	148	123	564
Numbers of fetuses with VSD (Dams with VSD)	4(4)	0	2(2)	2(2)	8
Incidence of VSD (% of fetuses)	2.9	0.0	1.4	1.6	1.42
Incidence of VSD (% of dams)	18.2	0.0	8.7	10.5	9.1

Signed : Shigeru Ishida Date: June 20 1996

Shigeru Ishida
Deputy director
Department I, Gotemba Laboratories
Bozo Research Center Inc.

Chemist Review

DIVISION OF NEUROPHARMACOLOGICAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-690 **CHEM.REVIEW #** 1 **REVIEW DATE:** 30-JUL-96

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
ORIGINAL	29-MAR-96	01-APR-96	01-APR-96

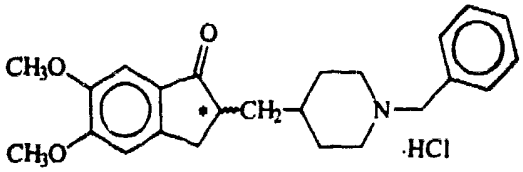
NAME & ADDRESS OF APPLICANT: Eisai America, Inc.
Glenpointe Centre East
Teaneck, NJ 07666-6741

DRUG PRODUCT NAME

Proprietary:	ARICEPT™
Nonproprietary/USAN:	Donepezil hydrochloride
Code Name/#:	E2020
Chem.Type/Ther.Class:	Reversible inhibitor of acetylcholinesterase
PHARMACOL.CATEGORY/INDICATION:	Treatment of Alzheimer's Disease
DOSAGE FORM:	Tablet
STRENGTHS:	5 mg and 10 mg (4.56 and 9.12 mg of donepezil free base)
ROUTE OF ADMINISTRATION:	Oral
DISPENSED:	XXXXX Rx_____OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOLECULAR WEIGHT:

(R,S)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methylpiperidine hydrochloride (IUPAC)



$C_{24}H_{29}NO_3.HCl$; Molecular Weight: 415.96;
CAS #: 14057-77-9

SUPPORTING DOCUMENTS: IND
RELATED DOCUMENTS:
REMARKS/COMMENTS: EISAI does not have its own manufacturing capability in the U.S. For that reason the NDS and the drug product will be manufactured by Pfizer Inc. in its Groton, CT and Brooklyn, NY facilities. To a lesser degree will help the sponsor as an alternate site for manufacturing of the drug product with 1/10th the capacity of Pfizer, Inc. The clinical studies were carried out with the donepezil hydrochloride tablets manufactured in Japan. Both sites: Pfizer's Brooklyn, N.Y., and facilities manufactured scale-up batches of donepezil hydrochloride tablets and placed them on stability studies. Both groups of batches were made with the NDS from Japan. Not one batch of tablets was produced using the NDS produced by Pfizer in Groton, CT. Dr S.W. Blum, the Team Leader, was alerted to the issue and discussed the issue with the sponsor copiously. A senior management decision has to be reached whether the Pfizer, Groton, CT facility should be approved as a manufacturing site for the NDS. The remainder of the deficiencies present in application is very easily to correct and does not impinge on the approvability of the application.

CONCLUSIONS & RECOMMENDATIONS: Recommend NDA 20-690: **APPROVABLE** subject to the positive inspection results of EISAI facilities in Japan and resolution of deficiencies listed in the Draft Letter.

cc: Orig. NDA 20-690
HFD-120
HFD-120/WJRzeszotarski
HFD-120/KHiggins
HFD-120/SWBlum
R/D Init by:SWB

W. Janusz Rzeszotarski, Ph.D., Chemist

filename: N020690.000

9/18/96

DIVISION OF NEUROPHAR[illegible]ACOLOGICAL DRUG PRODUCTS
Review of Chemistry, Manufacturing, and Controls

NDA #: 20-690 **CHEM.REVIEW #** 2 **REVIEW DATE:** 18-SEP-96

SUBMISSION TYPE:	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Amendment	05-APR-96	06-APR-96	06-APR-96
Amendment	05-JUN-96	06-JUN-96	06-JUN-96
Amendment	01-AUG-96	02-AUG-96	10-AUG-96
Amendment	04-SEP-96	05-SEP-96	05-SEP-96

NAME & ADDRESS OF APPLICANT: Eisai America, Inc.
Glenpointe Centre East
Teaneck, NJ 07666-6741

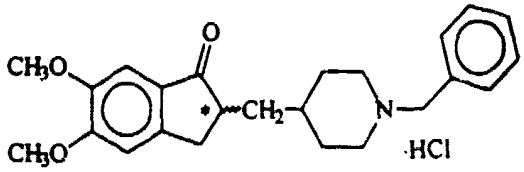
DRUG PRODUCT NAME
Proprietary: ARICEPT™
Nonproprietary/USAN: Donepezil hydrochloride
Code Name/#: E2020
Chem.Type/Ther.Class: Reversible inhibitor of acetylcholinesterase

PHARMACOL.CA[illegible]ORY/INDICATION: Treatment of Alzheimer's Disease
DOSAGE FORM: Tablet
STRENGTHS: 5 mg and 10 mg (4.56 and 9.12 mg of donepezil free base)
ROUTE OF ADMINISTRATION: Oral
DISPENSED: XXXXX Rx_____OTC

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR FORMULA, MOLECULAR WEIGHT:

(R,S)-1-benzyl-4-[(5,6-dimethoxy-1-indanon)-2-yl]-methylpiperidine hydrochloride (IUPAC)

$C_{24}H_{29}NO_3.HCl$; Molecular Weight: 415.96;
CAS #: 14057-77-9



SUPPORTING DOCUMENTS: IND
RELATED DOCUMENTS:
REMARKS/COMMENTS: The above listed amendments have been sub[illegible]tted to provide additional information in support of the aplication. In particular: 1) the comparative size of drug product biobatches from the primary (Pfizer, Inc. Brooklyn N.Y.) and secondary sites; 2) analytical data on four lots produced of drug substance produced at Pfizer, Inc. Groton, CT and comparative data on drug substance produced at Eisai Chemical Company in Kashima, Japan; and 3) additional stability data on drug substance produced at Pfizer, Groton, CT and drug product produced with that drug substance are provided. Attached is a copy of EER indicating that all facilities have been evaluated and are acceptable.
CONCLUSIONS & RECOMMENDATIONS: The data included in the above amendments supports the claim that the drug substance and the drug product manufactured in the sites indicated are identical
Recommend NDA 20-690, as amended: **APPROVABLE** subject to resolution of deficiencies listed in the Draft Letter.

W. Janusz Rzeszotarski, Ph.D., Chemist

cc: Orig. NDA 20 690
HFD-120
HFD-120/WJRzeszotarski
HFD-120/KHiggins
HFD-120/SWBlum
R/D Init by:SWB 9/18/96

filename: N020690.001

SENSITIVE

REVIEW

OF

ENVIRONMENTAL ASSESSMENT

FOR

NDA 20-690

ARICEPT™

(donepezil HCl)

Oral Tablets 5 and 10 mg

DIVISION OF NEUROPHARMACOLOGICAL DRUG PRODUCTS
(HFD-120)

CENTER FOR DRUG EVALUATION AND RESEARCH

DATE COMPLETED: July 23, 1996

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SUMMARY

A FONSI is recommended.

The EA qualifies for a Tier 0 EA review because the calculated EIC = 0.029 ppb.

Precautions taken at the sites of manufacture and methods of disposal may be expected to minimize occupational exposures and environmental release depending on the response to the deficiencies. The quantity of drug substance manufactured is estimated not to exceed 1300 kg in the fifth year of production after approval.

ENVIRONMENTAL ASSESSMENT

1. Date:

EA DATED February 8, 1996
EA signed March 1, 1996
CSO K. Higgins HFD-120
EA Review #1 initiated 7/8/96
EA Review #1 completed 7/23/96

2. Name of applicant/petitioner:

Eisai America, Inc.

3. Address:

Glenpointe Centre East
300 Frank E. Burr Blvd.
Teaneck, New Jersey 07666-6741

4. Description of the proposed action:

a. Requested Approval:

Applicant has filed a NDA for ARICEPT™ Film-Coated Tablets, 5 and 10 mg, and packaged in HDPE containers and blister packs.

Adequate.

b. Need for Action:

For treatment of symptoms of mild to moderately severe Alzheimer's disease.

Adequate.

c. **Production Locations:**

i. **Proprietary Intermediate(s):**

None

Adequate.

ii. **Drug Substance:**

Drug substance manufacture for the U.S.,

Pfizer Inc.
Groton, CT

Facility Description & Adjacent Environment:

The narrative description of the facility is given on page 345.

Adequate.

And

Eisai Chemical Co. Ltd.
Kashima, Japan

Facility Description & Adjacent Environment:

The narrative description of the facility is provided on page 346.

Adequate.

iii. **Finished Dosage Form:**
(Includes packaging)

Pfizer Inc.
Brooklyn, NY

Facility Description & Adjacent Environment:

The Pfizer Brooklyn facility is described on page 345.

Adequate.

And

Facility Description & Adjacent Environment:

The facility is described on page 346.

Adequate.

d. Expected Locations of Use (Drug Product):

Drug product will be used in home environments throughout the US.

Adequate.

e. Disposal Locations:

4.e.1. ***For Pfizer sites:***

Returned or outdated drug product, exclusive of samples will be disposed of by licensed high-temperature incineration. The facilities designated are annotated as CBI.

Adequate.

4.e.2. ***For Eisai sites:***

Waste/rejected drug substance/drug product and proprietary drug substance intermediates, where applicable, will be disposed-of by high temperature incineration at a licensed facility.

Adequate.

4.e.3. ***For Applied Analytical Industry site:***

Wastes will be disposed of by high temperature incineration (per confidential appendix 4).

Adequate.

5. Identification of chemical substances that are the subject of the proposed action:

Drug Substance:

USAN: donepezil hydrochloride

Code Name:

No code name is provided.

Chemical Name:

(±)-2,3-dihydro-5,6-dimethoxy-2-[[1-(phenylmethyl)-4-piperidinyl]methyl]-1*H*-inden-1-one, hydrochloride

CAS #:

[142057-77-0] & [120011-70-3]

Molecular Weight:

415.96

Molecular Formula:

$C_{24}H_{29}NO_3 \cdot HCL$

Structural Formula:

Structural formula provided on page 347

Physical Descrip.:

White to off-white solid.

Adequate.

Additives:

Confidential appendix 8 provides a list of additives with appropriate CAS numbers.

Adequate.

Impurities:

Total maximum impurities are ca. 1%. The EA should only identify impurities likely to be found in the described chemical substance at levels > than 1%.

Adequate.

6. Introduction of substances into the environment: For the site(s) of production:

a. Substances Expected to be emitted:

6.a.1. Drug Substance--Pfizer Groten Facility.

Substances expected to be emitted are presented in confidential appendix (CA) 6. There does not appear to be any substances which would adversely impact on the environment considering the small volume of drug substance manufacture.

Adequate.

6.a.2. Pfizer Brooklyn Facility (drug product)

There are no significant emissions from manufacture of the drug product into any environmental compartment.

Adequate.

Adequate.

6.a.4. Eisai Site:

Appendix 1 is a certification of compliance for foreign manufacture.

Adequate.

b. Controls (Air, Liquid Effluent, Solid):

6.b.1. Drug substance--Pfizer Groten Facility.

A general statement is provided that emissions controls are in place to ensure compliance with occupational exposure limits, general emissions limits, specific standards, permit limits and aquatic/terrestrial/atmospheric release environments. Organic-solvent-containing and other hazardous wastes show the permit numbers and deposition of the substances.

Adequate.

i. Air emissions are controlled by various filters and scrubbers/condensers. VOC emissions control device operates at > 93% efficiency.

Adequate.

ii. Aqueous emissions is comprised of equipment washings which undergo pre-treatment prior to disposal in a WWTF. They operate under a NPDES permit and achieve >95% reduction of influent process BOD.

Adequate.

iii. Terrestrial hazardous wastes are by licensed contract facilities by incineration. Nonhazardous wastes are handled by licensed disposal firms.

Adequate.

6.b.2. Pfizer Brooklyn Facility (drug product)

To ensure compliance with occupational exposure limits and general emissions requirements, emissions controls are used in the manufacturing processes.

i. Atmospheric--Particulates controlled using HEPA and similar filters with efficiencies > 99.9% and scrubbers.

Adequate.

ii. Aqueous-- Pretreatment of liquid discharges before disposal into a POTW is made in accordance with a NPDES permit.

Adequate.

iii. Terrestrial--Solid wastes disposal containing pharmaceuticals are by contract under appropriate permits.

Adequate.

6.b.3.

Adequate.

6.b.4. Eisai Facility

Appendix 1 is a certification of compliance for foreign manufacture.

Adequate.

c. Compliance with Federal, State and Local Emission Requirements:

6.c.1. Pfizer Groten Facility.

A certification statement is provided on page 349 of the EA along with a reference to applicable laws/regulations.

Adequate.

6.c.2. Pfizer Brooklyn Facility

A certification of compliance is found on page 350 of the EA along with a reference to applicable laws/regulations.

Adequate.

6.c.3.

Adequate.

6.c.4. Eisai Facility

Appendix 1 is a certification of compliance for foreign manufacture.

Adequate.

d. Effect of Approval on Compliance with Current Emissions Requirements:

6.d.1. Pfizer Groten Facility.

The firm states that approval will have a minimal effect upon compliance, i.e., permit parameters are estimated to be impacted < 3% of overall capacity.

Adequate.

6.d.2. Pfizer Brooklyn Facility

A discussion of the effect of approval on compliance is found on page 351 of the EA.

Adequate.

6.d.3.

Adequate.

6.d.4. Eisai Facility

Appendix 1 is a certification of compliance for foreign manufacture.

Adequate.

e. Estimated Expected Emitted Concentration/Quantities:

The $EIC_{aquatic}$ for donepezil hydrochloride is 0.032 ppb based on 5th year estimates of 1300 kg.

The $EIC_{aquatic}$ has been calculated (as donepezil) from the standard equation:

379.50/415.96 x 0.032 ppb = 0.029 ppb

The 29 pptr qualifies for the Tier 0 EA format.

Adequate.

7. **Fate of emitted substances in the environment:**

Not applicable for Tier 0 EA format. **Adequate.**

8. **Environmental effects of released substances:**

Not applicable for Tier 0 EA format. **Adequate.**

9. **Use of resources and energy:**

   a. **Natural Resources and Energy**
   b. **Effect on Endangered or Threatened Species**
   c. **Effect on property listed in or Eligible for Listing in the National Register of Historic Places**

Not applicable for Tier 0 EA format. **Adequate.**

10. **Mitigation measures:**

Not applicable for Tier 0 EA format. **Adequate.**

11. **Alternatives to the proposed action:**

Not applicable for Tier 0 EA format. **Adequate.**

12. **List of preparers, & their qualifications (expertise, experience, professional disciplines) and consultants:**

EA page 353 lists the preparers.

Adequate.

13. **Certification:**

The certification is signed and dated.

Adequate.

14. References:

Reference to the Industry Guidance document is made.

Adequate.

15. Appendices:

Four appendices are noted as non-confidential.

Eight appendices are noted as confidential.

Adequate.

DRAFT DEFICIENCY LETTER

No deficiencies of significance were noted. Therefore no deficiency letter is required.

Endorsements:

P. G. Vincent PGV 7/23/96

Nancy Sager 7/24/96

CC: Original NDA 20-690/HFD-120 K. Higgins copy to NDA 20-690
EA File 20690

C:\WPFILES\EAREVIEW\20690E01.RPV

ENVIRONMENTAL ASSESSMENT

AND

FINDING OF NO SIGNIFICANT IMPACT

FOR

ARICEPT™

(Donepezil HCl)

Oral Tablets 5 and 10 mg

NDA 20-690

Division of Neuropharmacological Drug Products
(HFD-120)

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

FINDING OF NO SIGNIFICANT IMPACT

for

ARICEPT™

(Donepezil HCl)

Oral Tablets 5 and 10 mg

NDA 20-690

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for ARICEPT™, Eisai America, Inc. has prepared an environmental assessment in accordance with 21 CFR 25.31a (attached) in the Tier 0 format which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Donepezil is a chemically synthesized drug which is administered as an oral tablet (5 and 10 mg) in the treatment of symptoms of mild to moderately severe Alzheimer's disease. The drug substance is manufactured by Pfizer, Inc., Groton, CT and Eisai Chemical Co. Ltd., Kashima, Japan. The finished dosage form (including packaging) is manufactured by Pfizer Inc., Brooklyn, NY and The finished drug product will be used in home environments throughout the United States.

Disposal of the drug may result from out of specification lots, discarding of unused or expired product, and user disposal of empty or partly used product and packaging. Returned or out-of-specification drug substance and rejected or returned drug product will be disposed of at some licensed high temperature incinerators. At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic regulations. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, while minimal quantities of unused

drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places

7/23/96
DATE

Approved
Phillip G. Vincent, Ph.D
Environmental Scientist
Center for Drug Evaluation and Research

7/24/96
DATE

Concurred
Nancy Sager
Acting Supervisor/Team Leader
Environmental Assessment Team
Center for Drug Evaluation and Research

Attachments: Environmental Assessment
Material Safety Data Sheet (drug substance)

END

BT

J.H.M. Research & Development, Inc., 5776 Second Street, N.E., Washington, D.C. 20011